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SECTION B: THE MICROBIOLOGY OF PRODUCTION OF HYDROCARBONS, LIPIDS AND RELATED BIOORGANICS

Methanogenesis in Arctic permafrost habitats

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Summary

In polar regions, huge layers of frozen ground, termed permafrost, are formed. Permafrost covers more than 25 % of the land surface and significant parts of the coastal sea shelves. Permafrost habitats are controlled by extreme climate and terrain conditions. Particularly, the seasonal freezing and thawing in the upper active layer of permafrost leads to distinct gradients in temperature and geochemistry. Methanogenic archaea in permafrost environments have to survive extremely cold temperatures, freeze-thaw cycles, desiccation and starvation under long-lasting background radiation over geological time scales. Although the biology of permafrost microorganisms remains relatively unexplored, recent findings show that methanogenic communities in this extreme environment are composed by members of the major phyla of the methanogenic archaea (*Methanobrevibacter*, *Methanobacterium*, *Methanosaeta*, *Methanosarcina*, *Methanolobus*/*Methano-halophylus*/*Methanococcoides*, *Methanculleus*/*Methanogenium*), with a total biomass comparable to temperate soil ecosystems. Currently, methanogenic archaea were the object of particular attention in permafrost studies, because of their key role in the Arctic methane cycle and consequently of their significance for the global methane budget.

Introduction

The Arctic plays a key role in the Earth's climate system for two reasons. On the one hand, global warming is predicted to be most pronounced at high latitudes, and observational evidence over the past 25 years suggests that this warming is already under way (Richter-Menge et al. 2006). On the other hand, one third of the global carbon pool is stored in ecosystems of the northern latitudes (Post et al. 1982, Zimov et al. 2006). Thus there is considerable socio-economic interest in predicting how the carbon balance of the northern ecosystems will respond to ongoing climate warming.
The degradation of permafrost and the associated intensified release of methane, a climate-relevant trace gas, represent potential environmental hazards (Anisimov et al. 1999). The microorganisms driving anaerobic carbon decomposition processes including methane production in Arctic permafrost environments have remained poorly investigated. Their population structure and reaction to environmental changes is largely unknown, which means that also an important part of the process knowledge on greenhouse gas fluxes in permafrost ecosystems is far from completely understood. This also hampers prediction of the effects of climate warming on Arctic methane fluxes. Further research on the stability of the methane cycling communities is therefore highly important for understanding the effects of a warming Arctic on the global climate (see also Chap. 23.b.i.5.a).
This chapter first gives an introduction into permafrost as an habitat for microorganisms. It then describes the current knowledge on the diversity and ecology of methane-producing archaea and gives an outlook for further research needs.

The permafrost environment

Permafrost, which covers about 25% of the Earth's land surface (Zhang et al. 1999) and significant parts of the coastal sea shelfs (Romanovskii et al. 2005), is defined as ground, comprised of soil or rock and included ice and organic material that remain at or below 0°C for at least two consecutive years (van Everdingen 2005). Arctic permafrost regions are characterized by low mean annual air temperatures (from -8 to -15°C), low mean annual precipitations (from 90 to 370 mm) and poor to missing vegetation (French 1996; ROSHYDROMET 2004). During the relatively short period of arctic summer, only the surface zone (a few dm-thick) of permafrost sediments thaws: this is called the *active layer*. Active layer depths range from a few cm in the high Arctic to more than 2 m in subarctic regions. Permafrost can be cemented by ice, which is typical for Arctic regions, or, in the case of insufficient interstitial water, may be dry as occurs in the Antarctic polar deserts or rocky areas.
The boundary between the active layer and the perennially frozen ground is called the *permafrost table*, which acts as a physical and chemical barrier. Intensive physico-chemical processes under extreme conditions take place in the active layer

and upper permafrost sediments (Ostroumov 2004). In the deeper permafrost layers, conditions have been stable for long periods of time and microbial processes are limited (Wagner, 2008).
Permafrost soils (*cryosols*) have been developed in the upper zone of the cryolithosphere (active layer and upper permafrost sediments) where the temperatures range from -50°C to +30°C (Yershov 1998). Therefore, permafrost soils are mainly formed by cryopedogenesis, which involves freezing and thawing, frost stirring, mounding, fissuring and solifluction. The repeating cycles of freezing and thawing leads to cryoturbation features (frost churning) that includes irregular, broken or involuted horizons and an enrichment in organic matter and inorganic compounds, especially on top of the permafrost table (Van Vliet-Lanoë 1991, Bockheim et al. 1999). As a result of cryopedogenesis, many permafrost soils are influenced by a strong micro-relief (e.g. low-centered ice-wedge polygons), which causes small-scale variations in soil types and vegetation characteristics (Kutzbach et al. 2004), as well as in the microclimatic conditions (Boike et al. 2008). This affects the abundance, processes and diversity of the methanogenic community in this habitat.

Methane cycle

The carbon pool estimates for permafrost soils vary between 190 Gt and, in more recent studies, approximately 900 Gt (Post et al. 1982, Anisimov and Reneva 2006, Zimov et al. 2006). These large variations can be attributed to different soil types (from mineral to peaty soils) and varying depths for the calculation (from the upper few centimeters to several m depth). The degradation of permafrost, therefore, could release large quantities of previously frozen organic matter. Permafrost degradation through environmental changes is considered to have a stronger impact on organic carbon decomposition rates than the direct effect of temperature rise alone (Eugster et al. 2000). This process is associated with the release of climate-relevant trace gases from intensified microbial carbon turnover that may further increase global warming and transform the Arctic tundra ecosystems from a carbon sink to a carbon source (Oechel et al. 1993).
However, permafrost soils can function as both a source and a sink for carbon dioxide and methane. Under anaerobic conditions, caused by flooding of the permafrost soils and the effect of backwater above the permafrost table, the mineralization of organic matter can only be realized stepwise by specialized microorganisms of the so called anaerobic food chain (Schink and Stams 2006). Important intermediates of the organic matter decomposition under anaerobic conditions are polysaccharides, low-molecular-weight organic acids, phenolic compounds and sugar monomers (Guggenberger et al. 1994; Kaiser et al. 2001). These compounds will be further converted for instance into hydrogen, carbon dioxide and acetate, which can be reduced to methane (methanogenesis) by

methanogenic archaea. The fermentation of carbon by microorganisms runs much slower than the oxidative respiration. As a result of the prolonged anaerobic conditions and low *in situ* temperatures of permafrost soils organic matter accumulates (peat formation) in these environments (sea above).
Wherever oxygen is present in permafrost habitats (upper oxic soil horizons, rhizosphere) methane can be oxidized to carbon dioxide by aerobic methane oxidizing bacteria (for details see Chapter 23.b.i.5.a).

Diversity and Ecology of methanogenic archaea in permafrost environments

Responsible for the biogenic methane production (methanogenesis) is a small group of microorganisms called methanogenic archaea (Garcia 1990). They can be found either in temperate habitats like paddy fields (Grosskopf et al. 1998), lakes (Jurgens et al. 2000, Keough et al. 2003), freshwater sediments (Chan et al. 2005), in the gastrointestinal tract of animals (Lin et al. 1997), or in extreme habitats such as hydrothermal vents (Jeanthon et al. 1999), hypersaline habitats (Mathrani and Boone 1985) or permafrost soils and sediments (Rivkina et al. 1998, Kobabe et al. 2004). Although methanogens are widely spread in nature they show an extremely specialized metabolism. They are able to converse only a limited number of substrates (e.g., hydrogen, acetate, formate, methanol, methylated amines) to methane. In cold environments such as permafrost two main pathways of energy-metabolism dominate: (i) the reduction of CO_2 to CH_4 using H_2 as a reductant and (ii) the fermentation of acetate to CH_4 and CO_2. In the case of CO_2-reduction organic carbon is not necessary for growth of methanogenic archaea (Conrad 2005).
At present, 26 genera with altogether 107 species of methanogenic archaea are described (http://www.ncbi.nlm.nih.gov/taxonomy). Genera with the most described species are *Methanobacterium*, *Methanobrevibacter*, and *Methanosarcina* (number of species between 9 and 14). Phylogenetically, they are classified as Archaea (Whitman et al. 2006), a group of microbes that are distinguished from Bacteria by some specific characteristics (e.g. cell wall composition, coenzymes). 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). Although, they are regarded as strictly anaerobic organisms without the ability to form spores or other resting stages, they are found in millions of years old permafrost sediments (Shi et al. 1997). 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). Although the metabolism of methanogenic archaea has been 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, e.g. subzero temperatures, low water activity and low nutrient availability (Vishnivetskaya et al. 2000, Høj et al. 2005, Ganzert et al. 2007).

Although permafrost environments are characterized by extreme climate conditions, it was recently shown that the abundance and composition of the methanogenic population is similar to that of communities of comparable temperate soil ecosystems (Wagner et al. 2005). The highest cell counts of methanogenic archaea were detected in the active layer of permafrost, with numbers of up to 3 x 10^8 cells g^{-1} soil (Kobabe et al. 2004). Methanogenic archaea represented between 0.5% and 22.4% of the total cell counts. Phylogenetic analyses revealed a great diversity of methanogens in the active layer, with species belonging to the families *Methanobacteriaceae*, *Methanomicrobiaceae*, *Methanosarcinaceae*, and *Methanosaetaceae* (Høj et al. 2005, Metje and Frenzel 2007, Ganzert et al. 2007). Other sequences detected were affiliated to the euryarchaeotal Rice Cluster II and V (Hales et al. 1996; Grosskopf et al. 1998, Ramakrishnan et al. 2001) as well as to the Group I.3b of the uncultured Crenarchaeota (non-methanogenic archaea; Ochsenreiter et al. 2003). Environmental sequences from the Laptev Sea coast form four specific permafrost clusters (Ganzert et al. 2007). Permafrost Cluster I was recovered mainly from cold horizons (with temperatures of less than 4°C) of the active layer and was related to *Methanosarcinacea*. Permafrost Clusters II and III were related to *Methanomicrobiales* and Permafrost Cluster IV was related to Rice Cluster II. It was hypothesized that these clusters comprise methanogenic archaea with a specific physiological potential to survive under harsh environmental conditions. The phylogenetic affiliation of the sequences recovered in the study by Ganzert and colleagues (2007) indicated that both hydrogenotrophic and acetoclastic methanogenesis exist in permafrost soils. Recent studies on perennially frozen permafrost deposits from the Lena Delta (Siberia) revealed significant amounts of methane which could be attributed to *in situ* activity of methanogenic archaea (Wagner et al. 2007). Another study on frozen ground on Ellesmere Island reported an archaeal community composed of 61% Euryarchaeota (i.a. methane producing archaea) and 39% Crenarchaeota, suggesting the presence of a diverse archaeal population also in the perennially frozen sediments (Steven et al. 2007). First studies on submarine permafrost sediments indicate a different methanogenic community in comparison to its terrestrial counterpart (Koch et al. 2009). Samples with high methane concentrations were dominated by sequences affiliated to the methylotrophic genera *Methanosarcina* and *Methanococcoides* as well as uncultured archaea.

So far, only a few psychrophilic and psychrotrophic strains as well as several uncultivated methanogens were obtained from Arctic and Antarctic habitats (Figure 1): *Methanococcoides burtonii* was isolated from the anoxic hypolimnion of the Ace Lake, Antarctica (Franzmann et al. 1992) and *Methanococcoides alaskense* was obtained from marine sediments from Skan Bay, Alaska (Singh et al. 2005). Both organisms are cold-adapted with a minimum temperature for growth of 1.7 and -2.3, respectively. Cells grew with trimethylamine as a catabolic substrate and some strains could also grow with methanol. *Methanogenium frigidum* was also isolated from the Ace Lake, Antarctica (Franzmann et al. 1997). The cells exhibiting most rapid growth at 15°C and no growth at temperatures above 20°C. The organisms grew by CO_2 reduction by using H_2 as a reductant. Formate could replace H_2, while acetate, methanol and trimethylamine were not catabolized. *Methanosarcina* spec. SMA-21, which is closely related to *Methanosarcina mazei*, was recently isolated from a Siberian permafrost soil in the Lena Delta. The organism grows well at 28°C and slowly at low temperatures (4°C and 10°C) with H_2/CO_2 as substrate. The cells grow as cocci, with a diameter of 1-2 µm. *Methanosarcina* SMA-21 is characterized by an extreme tolerance to very low temperatures (-78.5°C), high salinity (up to 6 M), starvation, desiccation and oxygen exposure (Morozova and Wagner 2007). Furthermore, this archaeon survived for three weeks under simulated thermo-physical Martian conditions (Morozova et al. 2007). Furthermore, five new strains of methanogenic archaea were isolated from permanently or periodically cold terrestrial habitats in Russia and Switzerland (Simankova et al. 2003). Three of them were members of the methylotrophic genus *Methanosarcina*, one hydrogenotrophic strain is a new ecotype of the genus *Methanocorpusculum* and one obligately methylotrophic strain is closely related to *Methanomethylovorans hollandica*. All new isolates are not true psychrophiles according to their growth temperature characteristics. In spite of the ability of all isolates to grow at temperatures as low as 1 to 5°C, all of them have their growth optima in the range of moderate temperatures (25 to 35°C). Thus, they can be regarded as psychrotolerant organisms.

Methane production was observed at low *in situ* temperatures with rates of up to 39 nmol CH_4 h^{-1} g^{-1} soil in the active layer of permafrost (Wagner et al. 2003, Høj et al. 2005, Metje and Frenzel 2007). The highest activities were thereby measured in the coldest zones of the profiles. Furthermore, it could be shown that methane production is rather limited by the quality of soil organic carbon than by the *in situ* temperature (Wagner et al. 2005, Ganzert et al. 2007). Another important factor affecting methanogenic communities in permafrost soils is the water regime. Along a natural soil moisture gradient, changes in archaeal community composition were observed, which suggest that the differences in these communities were responsible for the large-scale variations in methane emissions observed with changes in soil hydrology (Wagner et al. 2003, Høj et al. 2006).

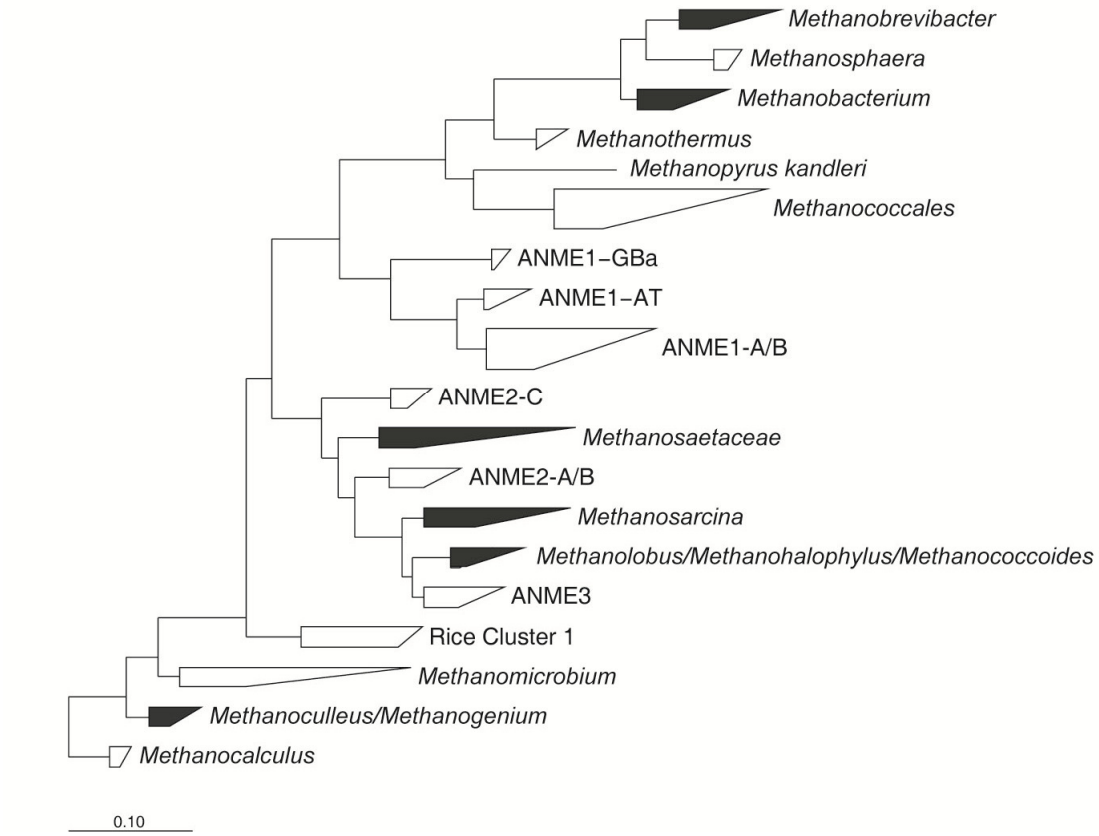

Figure 1: Phylogenetic relation (based on 16S rRNA gene sequences) of methanogenic archaea. Grey squares illustrate sequences with Arctic tundra origin (or groups containing sequences from Arctic tundra environments). Tree represent a maximum likelihood tree and were constructed using the ARB software package.

The permafrost environment forces the adaptation of the microbial communities to low temperature conditions and promotes the growth of species that so far remain undetected in temperate ecosystems. Therefore, Arctic permafrost environments can be seen as active microbial ecosystems rather than frozen habitats with microbial survivors. The evaluation of microbiological data and their correlation with climatic and geochemical results represents the basis for the understanding of the role of permafrost in the global system. Of particular relevance are feedback mechanisms related to nutrient cycles, biogeochemical processes and greenhouse gas emissions in the context of a warming Earth.

Research Needs

Although one fourth of the Earth land surface and distinct areas of the coastal sea shelfs are affected by permafrost, the physiology, function and diversity of microbial communities in these ecosystems is sparsely investigated so far. This may be

partially caused by the relative inaccessibility of the investigation areas and the associated logistic problems. However, the main difficulty lies in the lack of methodologies specific for permafrost sampling and isolation of cold-adapted microorganisms from Arctic soils and sediments. This is shown by the discrepancy between the small numbers of psychrophilic microorganism isolated so far from permafrost environments in contrast to the observed significant metabolic rates under *in situ* conditions. Methodological developments should consider the following aspects: enrichment of microorganisms should be performed directly in the field or in batch or continuous laboratory culture; culture techniques should be developed for the enrichment of "syntrophically associated" microorganisms; sub-zero culturing methods are needed; and state-of-the-art culture-independent molecular techniques for diversity and functional analyses of microbial communities should be applied on permafrost.

The lack of isolates from permafrost limits also possible biotechnological uses. Cold-adapted microorganisms from permafrost exhibit properties very different from those of other thermal classes. Therefore, the vast genetic resources of microorganisms from permafrost environments remain nearly unexploited. It is likely that mainly extremophilic microbes could offer technologically and/or economically significant products such as enzymes, polysaccharides, osmoprotectors and liposomes (Cavicchioli et al. 2002). Therefore, one essential goal of microbial diversity exploration in cold regions will be to recover new isolates, some of which will prove useful for biotechnology processes or medicine.

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