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**Handbook of hydrocarbon microbiology:
microbial interactions with hydrocarbons, oils, fats and related
hydrophobic substrates and products**

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**SECTION C: THE MICROBIOLOGY OF UTILIZATION OF HYDROCARBONS, OILS, LIPIDS
AND RELATED BIOORGANICS**

**Permafrost - Current and future challenges to study methanotrophy in
permafrost affected tundra and wetlands**

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Summary

Arctic permafrost affected wetlands and tundra are known to be sources of the greenhouse gas methane (Christensen et al. 1995, 2000, Wagner et al. 2003, Kutzbach et al. 2004, Sachs et al. 2008, Wille et al. 2008). As a consequence of the rising surface temperatures and the degradation of permafrost, methane emissions from these Arctic habitats are predicted to increase in the future. Evolution of methane fluxes across the subsurface/atmosphere boundary will thereby strongly depend on the activity of obligately aerobic methane oxidizing *Proteobacteria* which are known to be abundant and to significantly reduce methane emissions in permafrost affected soils despite seasonal freezing and thawing and despite the mostly extremely low temperatures. Based on their function as the major (known) sink for methane, research is needed on quantifying in-situ activities of aerobic methane oxidizing *Proteobacteria* and on understanding their potential to adapt to the currently changing environment. Also, possible unknown sinks for methane in these regions as for example known for marine methane rich habitats need to be explored.

Introduction

Methanotrophy, the consumption of methane, also occurs under the very harsh environmental conditions of cold environments (review by Trotsenko and Khmelenina 2005). Viable methane oxidizers were even detected in deep Siberian permafrost sediments with ages of 1000-100,000 years (Khmelenina et al. 2001). In permafrost affected tundra, methane is constantly bypassing the so called active layer during the period of Arctic summer (section B15.d.ii.). Wherever oxygen is present in these environments (elevated and dry sites, photosynthetically active polygonal ponds, rhizosphere), methane is oxidized to carbon dioxide. Though not yet quantified in-situ for permafrost affected soils, between 76 % and up to more than 90 % of the methane produced in wetlands gets oxidized before reaching the atmosphere (Roslev and King 1996, Le Mer and Roger 2001). During the last years, the group of microorganisms capable to consume methane (methanotrophs) has been observed to be very diverse comprising obligate aerobic members of the phyla *Proteobacteria* (Bowman 1999, 2006), and *Verrucomicrobiaea* (Dunfield et al. 2007; Pol et al. 2007), as well as anaerobically methane oxidizing *Archaea* in marine habitats (e.g. Boetius et al. 2000), and bacteria of a yet unknown phylum carrying out methane oxidation in the presence of very high nitrate and methane concentration in freshwater habitats (Raghoebarsing et al. 2006). In permafrost affected tundra, however, only aerobic methane oxidizers of the *Proteobacteria* phylum, described in detail elsewhere in this book (section C20c.i.) have been observed so far. Based on their pronounced distribution and function as the primary sink for methane in Arctic permafrost affected tundra (Wagner 2008), they are of major importance for the greenhouse gas (GHG) budget of these often methane rich environments.

Ecology of aerobic methanotrophic *Proteobacteria* in permafrost affected soils

The group of aerobic methanotrophic *Proteobacteria* comprises three families, *Methylococcaceae*, *Methylocystaceae*, and *Beijerinckiaceae* (Bowman 1999, Dedysh et al. 2000, 2001, 2002, 2004, Wise et al. 2001, Heyer et al. 2005, Tsubota et al. 2005, Rahalkar et al. 2007). *Methylococcaceae* include the genera *Methylobacter*, *Methylomonas*, *Methylomicrobium*, *Methylosarcina*, *Methylosphaera*, *Methylohalobius*, *Methylosoma*, *Methylothermus*, *Methylococcus*, and *Methylocaldum*. They belong to the gamma-subdivision of the *Proteobacteria* phylum and are termed type I methane oxidizing bacteria (MOB), except for the

last two which are also known as type X MOB. The families *Methylocystaceae*, and *Beijerinckiaceae* include the genera *Methylosinus*, *Methylocystis*, *Methylocella*, and *Methylocapsa*. Members of the *Methylocystaceae* and *Beijerinckiaceae* are termed type II MOB and belong to the alpha-subdivision of the *Proteobacteria* phylum.

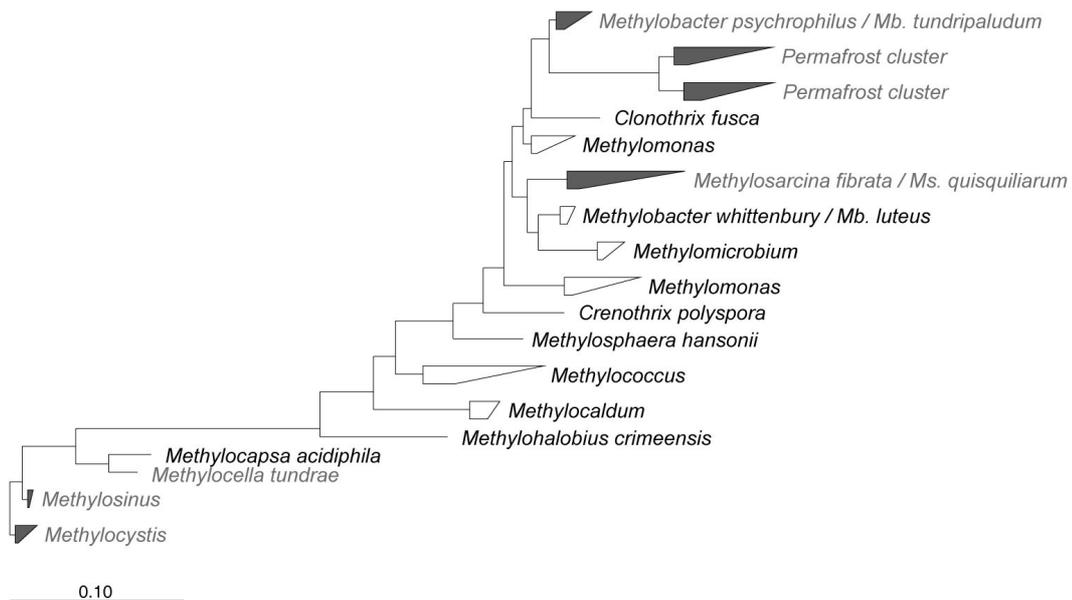


Figure 1: Phylogenetic relation (based on 16S rRNA gene sequences) of aerobic methanotrophic Proteobacteria. Grey squares illustrate sequences with Arctic tundra origin (or groups containing sequences from Arctic tundra environments). Trees represent maximum likelihood trees and were constructed using the ARB software package.

Almost 200 isolates of MOB are known (<http://www.ncbi.nlm.nih.gov/Taxonomy/>) suggesting that MOB are ubiquitous in many environments. Some psychrophilic and psychrotrophic strains as well as several uncultivated methanotrophs were obtained from Arctic permafrost habitats (Figure 1 of this chapter). *Methylobacter psychrophilus* (type I) is a true psychrophilic organism with optimum growth between 3.5 and 10 °C that was isolated from Siberian tundra (Omelchenko et al. 1996). *Methylobacter tundripaludum* (type I) with an optimum growth at 23 °C and *Methylocystis rosea* (type II) with an optimum growth at 27 °C are psychrotrophs isolated from Arctic wetland soils in Svalbard (Wartiainen et al. 2006a, b), and *Methylocella tundrae* (type II) with an optimum growth between 15

and 25 °C, as well psychrotrophic, was isolated from *Sphagnum* tundra peatlands (Dedysh et al. 2004). Additional psychrotrophic methanotrophs are *Methylosphaera hansonii* (type I) isolated from Antarctic, marine salinity, meromictic lakes (Bowman et al. 1997), and *Methylomonas scandinavica* (type I), isolated from deep igneous rock ground water (Kaluzhnaya et al. 1999). In general, type I methanotrophs were reported to dominate over the type II group in Arctic permafrost affected soils (Wartiainen et al. 2003, Wagner et al. 2005, Liebner and Wagner 2007).

Methane oxidizing *Proteobacteria* were shown to be highly abundant in permafrost soils of the Lena Delta, Siberia, with cell numbers ranging between 3×10^6 and 1×10^8 cells g^{-1} soil and contributing up to 10 % to the total number of microbial cells (Liebner and Wagner 2007). In the same area, specific clusters of methane oxidizing *Proteobacteria* closely related to *Methylobacter psychrophilus* and to *Methylobacter tundripaludum* were detected indicating a micro-diverse community on the species level (Liebner et al. in press). Highly divergent functional gene sequences of methanotrophs were found in soils of the high Canadian Arctic (Pacheco-Oliver et al. 2002). In contrast, the diversity of methane oxidizing *Proteobacteria* in wetland samples from Svalbard was observed to be restricted to only two genera (Wartiainen et al. 2003), whereas most methanotrophic *Proteobacteria* were detected in a Russian sub-arctic tundra (Kaluzhnaya et al. 2002). A recent study observed a shift between a mesophilic methanotrophic community near the surface and a psychrophilic methanotrophic community near the permafrost table of Siberian permafrost soils (Liebner and Wagner 2007). This indicates that depending on the environmental conditions both mesophilic as well as psychrophilic methanotrophs are active in Siberian permafrost soils. Still, it remains to be proven which group is responsible for methane oxidation at low and subzero temperatures in deep permafrost sediments (Trotsenko and Khmelenina 2005).

Methane oxidation in the context of thawing permafrost

Model calculations suggest that methane currently emitted from Arctic permafrost environments may enhance the greenhouse effect with a portion of approximately 20 % (Wuebbles and Hayhoe 2002). Arctic surface temperatures on average increased to a greater extent than those of the rest of the earth (IPCC 2001, 2007), causing a particular susceptibility of Arctic permafrost to degradation (Nelson et al. 2001). Global warming is assumed to degrade 25 % of the total permafrost area by 2100 (Anisimov et al. 1999). Serious concerns are

thus associated with the potential impact that thawing permafrost may have on the global climate system through release of greenhouse gases (Friborg et al. 2003, Christensen et al. 2004, Wagner et al. 2007) highlighting the importance of the current and of the future methane oxidation capacity in these regions. In models on modern methane emissions from Arctic wetlands, methane production and oxidation rates are primarily determined as functions of substrate availability, substrate concentration, and temperature as well as indirectly of water table and thaw depth (Walter et al. 2001, Zhuang et al. 2004, Anisimov 2007). Changes of these parameters will consequently lead to short-term alterations of methane production and methane oxidation rates. Whether, however, the currently observed climate change will effectively alter modern methane fluxes from Arctic permafrost affected wetlands will particularly depend on its long-term impact on the methane cycling communities and on their ability to adapt to the new environmental conditions. This ability is very likely dependant on the level of specialisation and diversity of the indigenous microbial communities. It was, for example, observed that an increase of temperature and precipitation altered the community structure and relative abundance of methane oxidizers in rice, forest and grassland soils (Horz et al. 2005, Mohanty et al. 2007). Also, the overall relative abundance and diversity of methanogenic archaea in a high Arctic peat from Spitsbergen increased with increasing temperature, in accordance with a strong stimulation of methane production rates (Høj et al. 2008). In contrast, the population structure of methanogenic archaea in a permafrost affected peat in Siberia remained constant over a wide temperature range (Metje and Frenzel 2007). There is, however, a lack of experimental research investigating the long-term effect of simulated climate change on the methane cycling communities in permafrost affected soils. Also, an account of the entire plant-microbe-animal-system and the interactions between metabolic networks, which are important for the production of methane (Panikov 1999), are missing in modern methane flux models.

Research needs

Though well known that methanotrophy by aerobic *Proteobacteria* functions as a major sink for methane in permafrost affected tundra and wetlands, experimental data on the actual amount of methane flux reductions by methanotrophs are completely lacking. In-situ rate measurements of methane oxidation activities such as push-pull-tests (e.g. Urmann et al. 2005, 2007, 2008) as well as below ground flux measurements would essentially improve our knowledge in this

context and would help to validate current methane flux models from Arctic wetlands. Also, as discussed in the introduction to this chapter, methanotrophy can be carried out by a variety of different bacterial phyla and even by members of the domain *Archaea*. So far the group of aerobic methanotrophic *Proteobacteria* is the only one known to be responsible for methane consumption in permafrost affected wetlands and tundra. Nevertheless, much more attention should be paid on exploring yet unknown communities possibly capable to consume methane in these habitats as well. These additional methane sinks might be of particular benefit considering how important the future methane oxidizing capacity will be in the scope of continuously degraded permafrost. We will finally have to investigate how methane consuming communities respond to (simulated or actual) global climate change. This includes a generally improved understanding on what determines the stability of methane oxidizing communities and what implications may arise from that knowledge to the future GHG budget of Arctic permafrost affected wetlands.

References

- Anisimov OA, Nelson FE, Pavlov AV (1999) Predictive scenarios of permafrost development under conditions of global climate change in the XXI century. *Earth Cryology* 3:15-25
- Anisimov OA (2007) Potential feedback of thawing permafrost to the global climate system through methane emission. *Environ Res Lett* 2:045016 (7pp)
- Boetius A, Ravensschlag K, Schubert CJ, Rickert D, Widdel F, Gieseke A, Amann R, Jørgensen BB, Witte U, Pfannuche O (2000) A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature* 407:623-626
- Bowman JP, McCammon SA, Skerratt MG (1997) *Methylosphaera hansonii* gen. nov., sp. nov., a psychrophilic, group I methanotroph from Antarctic, marine salinity, meromictic lakes. *Microbiology* 143:1451-1459
- Bowman JP (1999) The Methanotrophs – *The families Methylococcaceae and Methylocystaceae* In: Dworkin M (ed) *The Prokaryotes*, Springer, New York
- Bowman JP (2006) The Methanotrophs – *The families Methylococcaceae and Methylocystaceae*. *Prokaryotes* 5: 266-289

- Christensen TR, Jonasson S, Callaghan TV, Havström M (1995) Spatial variation in high-latitude methane flux along a transect across Siberian and Eurasian tundra environments. *J Geophys Res* 100:21035-21045
- Christensen TR, Friborg T, Sommerkorn M, Kaplan J, Illeris L, Soegaard H, Nordstroem C, Jonasson S (2000) Trace gas exchange in a high-arctic valley: 1. Variations in CO₂ and CH₄ flux between tundra vegetation types. *Glob Biogeochem Cyc* 14:701-713
- Christensen TR, Johansson TR, Akerman HJ, Mastepanov M, Malmer N, Friborg T, Crill P, Svensson BH (2004) Thawing sub-arctic permafrost: effects on vegetation and methane emissions. *Geophys Res Lett* 30:L04501
- Dedysh SN, Liesack W, Khmelenina VN, Suzina NE, Trotsenko YA, Semrau JD, Bares AM, Panikov NS, Tiedje JM (2000) *Methylocella palustris* gen. nov., sp. nov., a new methane oxidizing acidophilic bacterium from peat bogs, representing a novel subtype of serine-pathway methanotrophs. *Int J Syst Evol Microbiol* 50:955–969
- Dedysh SN, Horz HP, Dunfield PE, Liesack W (2001) A novel pmoA lineage represented by the acidophilic methanotrophic bacterium *Methylocapsa acidiphila* B2. *Arch Microbiol* 177:117–121
- Dedysh SN, Khmelenina VN, Suzina NE, Trotsenko YA, Semrau JD, Liesack W, Tiedje JM (2002) *Methylocapsa acidiphila* gen. nov., sp. nov., a novel methane-oxidizing and dinitrogenfixing acidophilic bacterium from Sphagnum bog. *Int J Syst Evol Microbiol* 52:251–261
- Dedysh SN, Dunfield PF, Derakshani M, Stubner S, Heyer J, Liesack W (2003) Differential detection of type II methanotrophic bacteria in acidic peatlands using newly developed 16S rRNA-targeted fluorescent oligonucleotide probes. *FEMS Microbiol Ecol* 43:299-308
- Dedysh SN, Berestovskaya YY, Vasilieva LV, Belova SE, Khmelenina VN, Suzina NE, Trotsenko YA, Liesack W, Zavarzin GA (2004) *Methylocella tundrae* sp. nov., a novel methanotrophic bacterium from acidic tundra peatlands. *Intern J Syst Evol Microbiol* 54:151-156
- Dunfield PF, Yuryev A, Senin P, Smirnova AV, Stott MS, Hou S, Ly B, Saw JH, Zhou Z, Ren Y, Wang J, Mountain BW, Crowe MA, Weatherby TM, Bodelier PLE, Liesack W, Feng L, Wang L, Alam M (2007) Methane oxidation by an extremely acidophilic bacterium of the phylum Verrucomicrobia. *Nature* 450:879-882
- Friborg T, Soegaard H, Christensen TR, Lloyd CR, Panikov N (2003) Siberian wetlands: where a sink is a source. *Geophys Res Lett* 30:2129

- Heyer J, Berger U, Hardt M, Dunfield PF (2005) *Methylohalobius crimeensis* gen. nov., sp. nov., a moderately halophilic, methanotrophic bacterium isolated from hypersaline lakes of Crimea. *Int J Syst Evol Microbiol* 55:1817-1826
- Horz HP, Rich V, Avrahami S, Bohannon BJM (2005) Methane-Oxidizing Bacteria in a California Upland Grassland Soil: Diversity and Response to Stimulated Global Change. *Appl Environ Microbiol* 71:2642-2652
- Høy L, Olsen RA, Torsvik VL (2008) Effects of temperature on the diversity and community structure of known methanogenic groups and other archaea in high Arctic peat. *ISME J* 2:37-48
- IPCC (2001) *Climate Change 2001: The Scientific Basis*. URL: http://www.grida.no/climate/ipcc_tar/wg1/index.htm
- IPCC (2007) *Climate Change 2007: Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, The Physical Basis of Climate Change*: <http://www.ipcc-wg2.org/index.html>
- Kaluzhnaya MG, Khmelenina VN, Kotelnikova S, Holmquist L, Pedersen K, Trotsenko YA (1999) *Methylomonas scandinavica* gen. nov., sp. nov., a new methanotrophic psychrotrophic bacterium isolated from deep igneous rock ground water of Sweden. *System Appl Microbiol* 22:565-572
- Kaluzhnaya MG, Makutina VA, Rusakova TG, Nikitin DV, Khmelenina VN, Dmitriev VV, Trotsenko YA (2002) Methanotrophic communities in the soils of the Russian northern taiga and subarctic tundra. *Microbiology* 71: 223-227
- Khmelenina VN, Makutina VA, Kaluzhnaya MG, Rivkina EM, Gilichinsky DA, Trotsenko YA (2001) Discovery of viable methanotrophic bacteria in permafrost sediments of northeast Siberia. *Dokl Biol Sci* 384:235-237
- Kutzbach L, Wagner D, Pfeiffer EM (2004) Effect of microrelief and vegetation on methane emission from wet polygonal tundra, Lena Delta, Northern Siberia. *Biogeochemistry* 69:341-362
- Le Mer J, Roger P (2001) Production, oxidation, emission and consumption of methane by soils: A review. *Eur J Soil Biol* 37:25-50
- Liebner S, Wagner D (2007) Abundance, distribution and potential activity of methane oxidizing bacteria in permafrost soils from the Lena Delta, Siberia. *Environ Microbiol* 9:107–117

- Liebner S, Rublack K, Stuehrmann T, Wagner D (in press) Diversity of aerobic methanotrophic bacteria in a permafrost soil of the Lena Delta, Siberia. *Microb Ecol* DOI: 10.1007/s00248-008-9411-x
- Metje M, Frenzel P (2007) Methanogenesis and methanogenic pathways in a peat from subarctic permafrost. *Environ Microbiol* 9:954-964
- Mohanty SR, Bodelier PLE, Conrad R (2007) Effect of temperature on composition of the methanotrophic community in rice field and forest soil. *FEMS Microbiol Ecol* 62:24-31
- Nelson FE, Anisimov OA, Shiklomanov NI (2001) Subsidence risk from thawing permafrost. *Nature* 410:889–890
- Omelchenko MB, Vasieleva LV, Zavarzin GA, Savelieva ND, Lysenko AM, Mityushina LL, Khmelenina VN, Trotsenko YA (1996) A novel psychrophilic methanotroph of the genus *Methylobacter*. *Microbiology* 65:339-343
- Pacheco-Oliver M, McDonald IR, Groleau D, Murrell JC, Miguez CB (2002) Detection of methanotrophs with highly divergent *pmoA* genes from Arctic soils. *FEMS Microbiol Lett* 209:313-319
- Panikov NS (1999) Understanding and predicting of soil microbial community dynamics under global change. *Appl Soil Ecol* 11:161-176
- Pol A, Heijmans K, Harhangi HR, Tedesco D, Jetten MSM, Op den Camp HJM (2007) Methanotrophy below pH1 by a new *Verrucomicrobia* species. *Nature* 450:874-878
- Raghoebarsing AA, Pol A, van de Pas-Schoonen KT, Smolders AJP, Ettwig KF, Rijpstra WI, Schouten S, Damsté JSS, Op den Camp HJM, Jetten MSM, Strous M (2006) A microbial consortium couples anaerobic methane oxidation to denitrification. *Nature* 440:918-921
- Rahalkar M, Bussmann I, Schink B (2007) *Methylosoma difficile* gen. nov., sp. nov., a novel methanotroph enriched by gradient cultivation from littoral sediment of Lake Constance. *Int J Syst Evol Microbiol* 57:1073-1080
- Roslev P, King GM (1996) Regulation of methane oxidation in a freshwater wetland by water table changes and anoxia. *FEMS Microbiol Ecol* 19:105-115
- Sachs T, Wille C, Boike J, Kutzbach L (2008) Environmental controls on ecosystem-scale CH₄ emission from polygonal tundra in the Lena River Delta, Siberia. *J Geophys Res* 113:G00A03
- Trotsenko YA, Khmelenina VN (2005) Aerobic methanotrophic bacteria of cold ecosystems. *FEMS Microbiol Ecol* 53: 15-26

- Tsubota J, Eshinimaev BT, Khmelenina VN, Trotsenko YA (2005) *Methylothermus thermalis* gen. nov., sp. nov., a novel moderately thermophilic obligate methanotroph from a hot spring in Japan. *Int J Syst Evol Microbiol* 55:1877-1884
- Urmann K, Gonzalez-Gil G, Schroth MH, Hofer M, Zeyer J (2005) New field method: Gas push-pull test for the in-situ quantification of microbial activities in the vadose zone. *Environ Sci Tech* 39:304-310
- Urmann K, Gonzalez-Gil G, Schroth MH, Zeyer J (2007) Quantification of microbial methane oxidation in an alpine peat bog. *Vadose Zone J* 6:705-712
- Urmann K, Schroth M, Noll M, Gonzalez-Gil G, Zeyer J (2008) Assessment of microbial methane oxidation above a petroleum-contaminated aquifer using a combination of in situ techniques. *J Geophys Res* 113:G02006
- Wagner D, Kobabe S, Pfeiffer EM, Hubberten (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 extreme permafrost habitats of the Lena Delta: effects of microbial community structure and organic matter quality. *Environ Microbiol*:1582-1592
- Wagner D, Gattinger A, Embacher A, Pfeiffer EM, 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 Biol* 13:1089-1099
- Wagner D (2008) Microbial communities and processes in Arctic permafrost environments. In: Dion P and Nautiyal CS (ed.) *Microbiology of extreme soils*, *Soil Biology* 13, Springer, Berlin, pp. 133-154
- Walter BP, Heimann M, Matthews E (2001) Modeling modern methane emissions from natural wetlands, 1. Model description and results. *J Geophys Res* 106:34,189-34,206
- Wartiainen I, Hestnes AG, Svenning MM (2003) Methanotrophic diversity in high arctic wetlands on the islands of Svalbard (Norway) — denaturing gradient gel electrophoresis analysis of soil DNA and enrichment cultures. *Can J Microbiol* 49:602-612
- Wartiainen I, Hestnes AG, McDonald IR, Svenning MM (2006a) *Methylobacter tundripaludum* sp. nov., a novel methanotrophic bacterium from Arctic wetland soil, Svalbard, Norway (78° N). *Intern J Syst Evol Microbiol* 56: 109-113

- Wartiainen I, Hestnes AG, McDonald IR, Svenning MM (2006b) *Methylocystis rosea* sp. nov., a novel methanotrophic bacterium from Arctic wetland soil, Svalbard, Norway (78° N). Intern J Syst Evol Microbiol 56:541-547
- Wille C, Kutzbach L, Sachs T, Wagner D, Pfeiffer EM (2008) Methane emission from Siberian Arctic polygonal tundra: eddy covariance measurements and modeling. Global Change Biol 14:1395-1408
- Wise MG, McArthur JV, Shimkets LJ (2001) *Methylosarcina fibrata* gen. nov. sp. nov. and *Methylosarcina quisquiliarum* sp. nov., novel type I methanotrophs. Int J Syst Evol Microbiol 51:611-621
- Wuebbles J, Hayhoe K (2002) Atmospheric methane and global change. Earth-Sci Rev 57: 177-210
- Zhuang Q, Melillo JM, Kicklighter DW, Prinn, RG McGuire, AD Steudler, PA Felzer, BS, Hu S (2004) Methane fluxes between terrestrial ecosystems and the atmosphere at northern latitudes during past century: A retrospective analysis with a process-based biogeochemistry model. Glob Biogeochem Cyc 18: 3010 (23pp)