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METHANOTROPHIC ABILITY OF MOSSES AND LICHENS ASSOCIATED BACTERIA IN PERMAFROST ECOSYSTEMS OF EASTERN SIBERIA

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ABSTRACT

Methanotrophic bacteria are unique group of microorganisms structurally and functionally adapted to use methane as a source of carbon, which is of great interest due to their ability to oxidize atmospheric methane. Methanotrophs are known to associate with mosses, which provide bacteria by habitat and protection. Methanotrophic bacteria provide mosses with carbon dioxide resulting of methane oxidation, whose content in moss tissues can reach 32%. We studied mosses and lichens sampled in Eastern Siberia permafrost ecosystems for methane oxidizing ability of associated bacteria, at concentrations of methane close to atmospheric. The consumption of methane in consortia of mosses and lichens and associated microorganisms was measured in laboratory incubation experiments. The methanotrophic activity registrated using gas analyzer Picarro 2201-i (Picarro Inc., USA) as a shift in the isotopic composition $\delta^{13}C$ in methane. It was shown that samples collected in permafrost soils have a larger ability to methanotrophy than sample collected in non-permafrost soils. In addition, we measured methanotrophic ability of the individual species of mosses and lichens. It has been shown that methanotrophs associated with lichens *Cladonia stelaris* and *Cetraria laevigata* have great methanotrophic ability on a level of methanotrophs associated with mosses *Rhytidium rugosum* and *Dicranum polysetum*.

Keywords: methanotrophic bacteria, permafrost ecosystems, mosses, lichens, $\delta^{13}C$ in methane

INTRODUCTION

Cryogenic ecosystems are the global carbon storage. In Russia, area underlain by permafrost is about 61%, and on the planet-wide scale, such ecosystems reach 25% [1]. Most of the buried organic carbon can be decomposed by microbes, in particular, by methanogenic microorganisms, resulting methane release into the atmosphere that will contribute to the greenhouse effect. Methane is the major end product of microbial carbon degradation in deeper, oxygen-free peat layers. A significant fraction of CH_4 ascending to the soil surface is oxidized before it escapes to the atmosphere by methane-oxidizing (methanotrophic) bacteria that thrive in soil layers where molecular oxygen is

available [2]. The oxidation of methane in high-latitude zones is directly related to the association of methanotrophic bacteria with mosses and lichens. It has previously been shown that mosses benefit from methane oxidation by additional CO_2 supply, but the benefit for methane-oxidizing bacteria in that association has not been proven, although increased oxygen supply from photosynthesis and stabilization of bacterial cells in the aerobic-anaerobic interface has been suggested [3].

In recent years, stable isotope analysis has become a powerful tool particularly for environmental scientists to track the complimentary processes of methanogenesis and methanotrophy and to study the global CH_4 cycle. Most chemical and biochemical reactions show a slight preference for one isotope of an element over another, usually because of their different masses. The partitioning of the light and heavy isotopes of hydrogen and carbon and the resultant isotope signatures (^{13}C and ^{2}H values) can be diagnostic for the identification of CH_4 origin and pathway. Furthermore, isotope-labelling experiments of potential organic precursor molecules are often applied to finally verify hypothetic reaction pathways [4]. Isotopic methods have been developed on the preference of methanotrophs for the isotope of smaller mass, ^{12}C rather than ^{13}C, according to one or more fractionation factor(s) dependent on soil properties and gaseous transport considerations [5], [6], [7]. Methanotrophic bacteria will oxidize $^{12}CH_4$ at a slightly more rapid rate than $^{13}CH_4$. In general, ^{13}C values for unoxidized CH_4 in the anaerobic zone range from about –57 to –60; with oxidation, these values can undergo a positive shift to –35 or more [8].

The methane filter consisting of aerobic methanotrophic bacteria acts as a natural barrier capable of significantly reducing the methane flux into the atmosphere [9]. Most studies of methanotrophic communities of tundra wetlands published so far have been performed in peat bogs dominated by *Sphagnum* mosses. However, tundra landscapes commonly feature discontinuous permafrost wetlands with a mosaic cover of *Sphagnum* mosses and lichens of the genera *Cladonia, Cetraria*, and others (reindeer lichen). Although tundra lichens were investigated in a considerable number of studies, little is known about the composition and activity of associated methanotrophic communities [10].

We studied mosses and lichens sampled in Eastern Siberia permafrost ecosystems (forest and tundra) for methane oxidizing ability of associated bacteria, at concentrations of methane close to atmospheric in application to ecological conditions.

STUDY SITES AND METHOD DESCRIPTION

In our research, we studied mosses and lichens sampled in Baikal region in permafrost and non-permafrost sites; and mosses and lichens sampled on Samoylov Island, Lena river delta, area underlain by continuous permafrost.

The consumption of methane in consortia of mosses and lichens and associated microorganisms was measured in laboratory incubation experiments. Samples of mosses or lichens were placed in separate gas-tight plastic boxes of 1500 ml volume. Boxes were hermetically sealed. Immediately after placing sample in the box and closing, we measured initial concentrations of CH_4 and $\delta^{13}C$ in methane using Picarro G2201-i gas analyzer (Picarro, Inc., United States). The following measurements of CH_4

concentrations were made in 4 hours and 24 hours. The methanotrophic activity controlled by a shift in the isotopic composition $\delta^{13}C$ in methane.

Rate of the CH_4 consumption (mg CH_4 g^{-1} h^{-1}) was calculated based on the changes of its concentration in boxes after 24 hours of incubation at 23°C according the following equation:

$$R_{CH4}= \Delta CH_4/\Delta t*(V_{air}*M_{CH4})/22.41*273.15/T_{air}*1/W_{sample} \quad (1),$$

where R_{CH4} is sample methane consumption (mg CH_4 g^{-1} h^{-1}), $\Delta CH_4/\Delta t$ is the CH_4 concentration increment (ppm) per unit time (h), V_{air} is the air volume (liter) in the chamber calculated as $V_{air} = V_{chamb} - V_{sample}$ (V_{chamb} – volume of the chamber, V_{sample} – volume of sample), M_{CH4} is the molar mass of CH_4 (16 g mol^{-1}), T_{air} is the air temperature (K), 22.41 is the molar volume (L mol^{-1}) at standard temperature (273.15 K) and pressure (1.013 bar), and W_{sample} is the sample dry weight (g) [11].

RESULTS

In the incubation experiments with mosses and lichens consortia collected on permafrost and non-permafrost sites was shown that samples collected in permafrost soils have a larger ability to methanotrophy than samples collected in non-permafrost soils (Tabl. 1). This observation was made according to the shift in the isotopic composition $\delta^{13}C$ in methane. For example, $\delta^{13}C$ in methane of mosses consortia from permafrost soils of 1 and 3 sites was 156 ‰ and 118 ‰, respectively, that showed a large ability of these consortia to methane consumption.

Table 1

Methane consumption dynamic and $\delta^{13}C$ in methane emitted by mosses and lichens consortia of studied areas.

Studied areas*	Samples	CH_4, ppm			$\delta^{13}C$-CH_4, ‰		
		0 h	4 h	24 h	0 h	4 h	24 h
2	Lichens from non-permafrost soils	1,97	1,96	2,00	-53	-31	-15
2	Mosses from non-permafrost soils	1,97	1,95	1,88	-57	-30	-19
1	Mosses from permafrost soils	1,97	1,98	1,90	-47	-14	156
3	Mosses from permafrost soils	1,97	1,96	1,93	-51	-22	118
3	Lichens from permafrost soils	1,97	1,98	1,90	-54	-42	-26
4	Mosses from permafrost soils	1,97	1,89	1,90	-48	-40	-30
1	Epiphytes	1,97	1,93	1,54	-46	15	205

** Sites 1,3,4 located on permafrost soils, site 2 located on the non-permafrost soils*

Epiphytes sampled from trees growing in permafrost sites also showed large ability for methane consumption, about 205 ‰ to 24 h of incubation. Ability for methane consumption of lichens and mosses consortia sampled in non-permafrost soils was much lower in comparison to consortia growing in permafrost ecosystems.

In every collected consortia we identified dominant species of mosses and lichens. In general, eight species were identified: *Dicranum polysetum, Pleurozium schreberi, Cladonia rangiferina, Cladonia arbuscula, Cetraria laevigata, Rhytidium rugosum, Dicranium sp.* Some of identified species were presented in several collected consortia, data are shown in Table 2.

Table 2

Dominant species of mosses and lichens identified in studied samples and their presence in consortia

Species / Sample, description	*Dicranum polysetum*	*Pleurozium schreberi*	*Cladonia rangiferina*	*Cladonia arbuscula*	*Cladonia stelaris*	*cCetraria laevigata*	*Rhytidium rugosum*	*Dicranium sp*
Lichens on non-permafrost soils			+	+	+			
Mosses on non-permafrost soils	+	+						
Mosses on permafrost soils	+							
Mosses on permafrost soils	+	+						
Lichens on permafrost soils				+		+		+
Mosses on permafrost soils		+					+	

For every identified species of mosses and lichens, methanotrophic ability in incubation experiments (as described above) was made. Some separated results of the experiments presented in Table 3. It was shown, that lichens *Cladonia stelaris* and *Cetraria laevigata* have large methanotrophic ability on a level with mosses *Rhytidium rugosum* and *Dicranum polysetum*. Special emphasis should be paid to *Cetraria laevigata* due to methanotrophic ability more than 10 times higher than the other mosses and lichens investigated.

Table 3

Methane consumption dynamic and $\delta^{13}C$ in methane emitted by individual species of mosses and lichens consortia of studied areas

Studied areas*	Samples	CH_4, ppm			$\delta^{13}C$-CH_4, ‰		
		0 ч	4 ч	24 ч	0 ч	4 ч	24 ч
2	*Cladonia stelaris*	1,90	1,83	1,82	-41	-11	-5
3	*Cetraria laevigata*	1,90	1,07	1,12	73	780	6900
4	*Rhytidium rugosum*	1,90	1,92	1,88	-60	-58	-47
2	*Dicranum polysetum*	1,90	1,84	1,81	-59	-55	-42
1	Epiphytes	1,90	1,88	1,87	-56	-58	-22
	Blanc	2,00	2,00	1,91	-50	-51	-52

** Sites 1,3,4 located on permafrost soils, site 2 located on the non-permafrost soils*

DISCUSSION

To compare the activity of methane-oxidizing filters associated with different consortia of the studied sites, we calculated a potential rates of methane oxidation by samples and individual mosses and lichens species. We showed that rates at the lichens dominated sites were significantly lower than at the mosses-dominated sites. The values of potential methane oxidation rates in samples collected permafrost sites were comparable to the rates in samples collected in non-permafrost sites. Data obtained by Danilova and Dedysh [12] for the peat of acidic *Sphagnum* bogs of the European part of Russia showed that an efficient methaneoxidizing filter was active in the *Sphagnum*–lichen tundra wetland studied, and that its activity was the highest at lichen-dominated sites. In contrast, in our experiments with forest mosses and lichens consortia collected in forest ecosystem (Baikal region), mosses-dominated sites showed larger methane oxidation potential.

Other researchers also described the high methanotrophic activity of bacteria associated with *Sphagnum*-mosses in peat soils. Stępniewska et al. [13] detected several strains of endophytic methanotrophic bacteria of *Sphagnum magellanicum*. By the 16S r-RNA sequencing, the strains were identified as species of the genus *Methylomonas*. Their methanotrophic activity reaches 67.55 μM CH_4 $g^{-1}day^{-1}$ under laboratory conditions. At the same time, endosymbionts associated with plants of the family *Cyperaceae* exhibited lower methanotrophic activity (0.865 to 22.90 μM CH4 $g^{-1}day^{-1}$). Putkinen et al. [14] also indicated a high potential of sphagnum *Sphagnum*-mosses for CH_4-oxidation and, as a result, their valuable role as a methane biofilter. These data are confirmed by the presence of functional genes of methanotrophic bacteria detected using the pmoA-gene targeting microarray method.

Despite of it, two separated lichen species *Cladonia stelaris* and *Cetraria laevigata* in our experiments demonstrated the highest potential rates of methane oxidation that confirm the data obtained by [10], [12].

Recent studies show that methane filter bacteria play an important role not only in marshlands [15]. Natural soils have a high methane absorption potential even after anthropogenic impact or conversion to agriculture. At the same time, canonical high-affinity methanotrophs capable of oxidizing (circum-)atmospheric methane may also utilize methane at higher concentrations upon availability. Thus, the authors point to an insufficient assessment of the contribution of canonical methanotrophs to methane absorption.

Based on our obtained results and results obtain by [10], [12] [15]. we concluded that microorganisms constituting the methane-oxidizing filter of subarctic tundra and forest ecosystems currently remain poorly studied.

CONCLUSION

Studied mosses and lichens sampled in Eastern Siberia permafrost ecosystems showed pronounced methane oxidizing ability of associated bacteria, at concentrations of methane close to atmospheric. The consumption of methane in consortia of mosses and lichens and associated microorganisms was measured in laboratory incubation experiments. The methanotrophic activity registrated using gas analyzer Picarro 2201-i (Picarro Inc., USA) as a shift in the isotopic composition $\delta^{13}C$ in methane. It was shown

that samples collected in permafrost soils have a larger ability to methanotrophy than sample collected in non-permafrost soils. In addition, we separated all samples into individual species of mosses and lichens and measured methanotrophic ability of the species. In general, eight species were identified. It was shown, that lichens *Cladonia stelaris* and *Cetraria laevigata* have large methanotrophic ability on a level with mosses *Rhytidium rugosum* and *Dicranum polysetum*. Special emphasis should be paid to *Cetraria laevigata* due to methanotrophic ability more than 10 times higher than the other mosses and lichens investigated.

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