METHANOTROPHS RESPONSIBLE FOR METHANE OXIDATION IN NATURAL PEATS FROM POLESIE LUBELSKIE REGION [*]

Anna Szafranek-Nakonieczna, Zofia Stępniewska, Agnieszka Wołoszyn, Jakub Ciepielski

Department of Biochemistry and Environmental Chemistry,
The John Paul II Catholic University, Al. Kraśnicka 102, 20-718 Lublin, Poland
e-mail: anna.szafranek@kul.lublin.pl

Abstract. The potential of methanotrophic activity (MTA) has been investigated under laboratory conditions in three types of peatland profiles: high (H), transition (T) and low (L) originating from Polesie Lubelskie Region. Selected peat samples differed in respect of pH, TOC, von Post index and moisture. The experiment was conducted at natural moisture (198-719 %w/w) with different ranges of both, temperature (5, 10 and 20°C) and CH_4 enrichment (1 and 5%v/v). The highest MTA (19.69-155.79 mg CH_4kg D.W.$^{-1}$ d^{-1}) was observed at 20°C. Regardless of temperature, MTA was lower (1.38-51.16 mg CH_4 kg D.W.$^{-1}$ d^{-1}) when peat samples were incubated in atmosphere enriched in 1% than in 5% CH_4 v/v (4.75-191.26 mg CH_4kg D.W.$^{-1}$ d^{-1}). Strong influence of temperature and sampling sites on MTA was also noted. Total DNA was isolated from the most active (20°C, 5% CH_4 v/v) peat samples from each site and the PCR (polimerase chain reaction) amplifying of genes *pmoA* (primers A189f/mb661r) and sequence 16S rRNA (primers Type If /Type Ir and Type IIf/Type IIr) specific for methanotrophic bacteria were carried out. Positive results of PCR with primers of *pmoA* gene after sequencing confirmed that methanotrophs from L point belong to family *Methylococcaceae*, while 16S rRNA gene sequences from microorganisms inhabiting H peat demonstrated the highest similarity to genus *Methylocystis* and *Methylosinus*.

Keywords: methanotrophic activity, methanotrophs, peat soil

INTRODUCTION

Methanotrophs, microorganisms involved in aerobic methane oxidation, focused a great interest of scientists and thus extended our knowledge about global methane cycling. Those bacteria grow on methane as a sole source of carbon and

---

energy. The important role of methanotrophs in the environment results from the fact that up to 90% of methane produced by methanogens (methane producing *Archaea*) may be oxidised by methanotrophs before its release into the atmosphere. Biological methane oxidation is found in many places such as wetlands, soils, freshwaters, sediments, rice plantations and some extreme environments (Chen and Murrell 2010). A large group of environments favourable for methanotrophs are peatlands, especially their aerobic parts.

Methanotrophs can be broadly divided into Type I (*Gamma-Proteobacteria*) and Type II (*Alpha-Proteobacteria*). The first can be further subdivided into two classes: Type Ia and Type Ib, also referred to as Type X (Dumont *et al.* 2011). The types of methanotrophs differ in many characteristics including (a) carbon assimilation pathways (Type Ia uses ribulose monophosphate pathway, Type Ib ribulose monophosphate or serine pathway, while Type II uses only serine pathway) and (b) the arrangement of intracellular membranes (Heyer *et al.* 2002, Murrell 2010). Type Ia is represented by genera *Methylococcus, Methylocaldum, Methylomonas, Methylomicrobium, Methylobacter, Methylosphaera, Methylosarcina*. Type Ib contains the genera of *Methylocaldum* and *Methylococcus,* while genera of *Methylocystis, Methylosinus, Methylocella*, and *Methylocapsa* are representatives of Type II (Dumont *et al.* 2011).

Even though the Type I group is more phylogenetically diverse, the members of Type II are more often isolated from natural systems, or detected using cultivation independent molecular techniques (Heyer *et al.* 2002). Quantitative studies suggest that methanotrophic microorganisms from *Alpha-Proteobacteria* are numerically predominant also in peatlands (Dunfield and Dedysh 2010).

Methane is converted by methanotrophs to methanol through catalysis by the methane monooxygenase enzyme (MMO), subsequently oxidised by methanol dehydrogenase, formaldehyde dehydrogenase and formate dehydrogenase to produce CO_2 and H_2O. Generally, methanotrophs can express a membrane-bound MMO termed particulate methane monooxygenase (pMMO), however when copper ion is unavailable, some strains are able to express an iron-containing soluble methane monooxygenase (sMMO) to catalyse the hydroxylation of methane to methanol (Hua *et al.* 2007). The pMMO is therefore the universal enzyme of all methanotrophs, and recognised genes encoding that enzyme are used as phylogenetic markers. One of them is the *pmoA* gene which encodes the active-site-containing subunit of the particulate methane monooxygenase enzyme (Heyer *et al.* 2002). The next most obvious marker for detecting methanotrophs in various environments is the 16S rRNA gene, due to the large database of sequences available.

In relation to preferred methane concentration, methanotrophs are divided into two groups: methanotrophs that can survive under atmospheric concentrations of methane (high affinity methanotrophs) and methanotrophs surviving only in methane presence at >40 ppmv (low affinity methanotrophs) (Chen and Murrell 2010).

The aim of the present work was examination the peat soil profiles (0-80 cm) originating from tree different types of peatland characteristic for Poland in respect of their ability of methane oxidation. The second objective was an attempt to identify methanotrophs responsible for these activities. The sequences of *pmoA* and 16S rRNA genes specific primers were used for amplification.

MATERIALS AND METHODS

Soil characteristics

The three plots used in this study were selected in Polesie Lubelskie Region (Eastern Poland). The first site, defined as a low moor peatland (L) with numerous pools, fed by shallow groundwater was situated west of Garbatówka village (51°21' N, 23°7' E). Its vegetation mainly consisted of *Carex* spp., *Juncus* spp*., Schoenoplectus* spp. and various mosses.

The next studied point (51°25' N, 23°3' E) was placed close to Orłowskie Peatland, representing a transition moor peat (T), fed by ground water and rainfalls, and adjoining with Lake Łukie. Plant cover was represented by *Poa pratensis, Festuca rubra, Deschampsia caespitose, Rumex acetosa* and locally shrubs with *Salix lapponum* and *Salix myrtilloides*.

The last site (51°27' N, 23°6' E), a high moor peat (H), was located on the south of Lake Moszne, and characterised by lacustrine genesis, with water supply only by precipitation. Therefore, in hot and drought seasons the groundwater table considerably decreases. The vegetation was composed of *Vaccinium uliginosum, Ledum palustre, Eriophorum vaginatum,* a great number of peat mosses (e.g. *Sphagnum acutifolium*, *Sphagnum magellanicum*), shrubs and trees: *Pinus sylvestris, Betula pubescens, Quercus robur*, *Populus tremula*, *Betula pendula* (Wojciechowski and Szczurowska, 2002; Radwan, 2003). Additional soil characteristics are presented in Table 1.

Soil moisture was determined gravimetrically by drying peat samples at 105°C (24h). Soil pH was determined potentiometricaly, in soil–water mixture (1/1, w/w) using a pH electrode and pIONeer 65 meter (Radiometer Analytical S.A., France). Total organic carbon was determined in dry peat samples by combustion and the analysis of evolved CO_2 by means of TOC-VCSH with module SSM-5000A (Shimadzu, Japan).

Methane and oxygen concentrations were measured using a gas chromatograph (GC 3800, Varian, USA) equipped with flame ionisation (FID) and thermal conductivity (TCD) detectors, according to methods described by Szafranek-Nakonieczna and Bennicelli (2010).

Table 1. Characteristic of investigated peat materials, ±SD

Site	Peat layer (cm)	pH (H_2O)	Von Post ranking (%)	Moisture (% w/w)	Bulk density (g cm^{-3})
	0-20	6.93±0.02c	H_2a	592.9±0.1c	0.708±0.02a
	20-40	6.80±0.02b	H_2a	530.1±0.1a	0.743±0.03b
L	40-60	6.83±0.02b	H_3b	582.4±0.2b	0.859±0.05c
	60-80	6.75±0.02a	H_3b	667.1±0.3d	0.879±0.01c
	0-20	6.17±0.02a	H_4b	284.1±0.1a	1.05±0.01a
	20-40	6.44±0.02b	H_5b	400.0±0.1b	1.055±0.01a
T	40-60	6.52±0.02c	H_6c	563.2±0.1c	1.189±0.03b
	60-80	6.53±0.02c	H_7d	573.1±0.1d	1.227±0.06b
	0-20	2.87±0.01a	H_3a	198.1±0.2a	0.578±0.02a
	20-40	2.98±0.01b	H_4b	315.2±0.1b	0.717±0.04b
H	40-60	3.36±0.01c	H_5c	667.1±0.2c	0.736±0.01b
	60-80	4.43±0.01d	H_6d	719.1±0.1d	0.824±0.01c

- numbers characterising the properties of peat from the one location, followed by the same letter, do not differ significantly (*P*>0.05).

Incubation experiments

The effects of elevated levels of CH_4 (1 and 5% CH_4 v/v), temperature (5, 10, 20°C) and position in peat profile (from top down to 80 cm) on the potential of peat for methane oxidation were tested via laboratory incubation experiments. The peat samples were collected from separated layers (0–20, 20-40, 40-60 and 60-80 cm) of plots L, T and H. Next, they were placed (10 g of fresh peat) in dark incubation bottles (60 ml), tightly sealed with rubber plug and aluminium cap. For each combination three replicates were prepared. Next, the headspace of each unit was enriched with methane (1 or 5% CH_4 v/v). Incubation was performed until substrate depletion. At the end of the incubation, MTA was calculated and expressed as mg CH_4 kg D.W.$^{-1}$ d^{-1}.

DNA extraction and electrophoretic check of the amount of isolated DNA

Total DNA was extracted from the samples (0.5 g of peat soil) selected from material the most active in methane oxidation. The extraction procedure was performed stepwise according to the method described by Sambrook and Russel (2001).

Electrophoresis was performed in agarose gel 1% (w/v) in 1-fold concentrated TAE buffer and 1-fold TAE buffer, at a voltage of 5 V/cm of distance between the electrodes. As a marker, DNA Ladders MassRuler were used. The gel was stained in 0.01% aqueous solution of ethidium bromide and observed under UV lamp (Vilber Laurmat, TFX-20M, 6x15W-312 nm tube, France) (Sambrook and Russel 2001).

PCR reaction

In order to confirm the presence of the *pmoA* gene encoding the active site of subunit molecular methane monooxygenases (pMMO) a universal enzyme of methanotrophs and 16S rRNA gene sequences characteristic for methanotrophs of Type I and Type II, pairs of primers were used (as is shown in Table 2).

Table 2. Primers used in the study

Target gene	Primer	Sequence (5'-3')	References
pmoA	A189f	GGN GAC TGG GAC TTC TGG	Holmes *et al.* 1996 Liebner *et al.* 2009
	mb661r	CCG GMG CAA CGT CYT TAC C	Costello and Lidstrom 1999 Liebner *et al*. 2009
16S rRNA methanotrophs Type I	Type IIf	GGG MGA ATA ATG ACG GTA CCW GGA	McDonald *et al*. 2008
	Type IIr	GTC AAR AGC TGG TAA GGT TC	McDonald *et al*. 2008
16S rRNA methanotrophs Type II	Type If	ATG CTT AAC ACA TGC AAG TCG AAC G	McDonald *et al*. 2008 Chen *et al.* 2007
	Type Ir	CCA CTG GTG TTC CTT CMG AT	McDonald *et al.* 2008 Chen *et al.* 2007

Polymerase chain reaction (PCR) amplifications were performed in 50 µl (total volume) using PCR master Mix (Fermentas) with 3.5 µl of template DNA in MJmini thermal cycler (Bio-Rad). Amplification was carried out as follows: initial denaturation (96°C for 4 minutes), followed by 30 cycles of denaturation (94°C for 2 min), primer annealing (56°C for Type I, 55°C for Type II and 53°C for *pmoA* each for 1 min), elongation (72°C for 1 min). Final elongations at 72°C were performed for 3 minutes (Sambrook and Russel 2001).

Sequencing

The sequencing of PCR products was performed in the Laboratory of DNA Sequencing and Oligonucleotide Synthesis of the Institute of Biochemistry and Biophysics, Polish Academy of Science, in Warsaw. The sequencing of PCR products were carried out with pairs of primers used for amplification.

Analysis of similarities

In order to determine the similarity of sequences of obtained fragments *pmoA* and 16S rRNA genes with other known methanotrophic microorganisms, interactive tool from the NCBI, BLASTN was used (Blast Local Alignment Search Tool).

RESULTS

Methane consumption ranged from 1.38 to 191.3 mg CH_4 kg D.W.$^{-1}$ d^{-1} in the aerobic treatments. The highest oxidation was observed in the peat from site H, in the layer of 40-60 cm, incubated at 20°C. Horizontal distribution of MTA in all investigated peat profiles was observed. It varied depending on substrate availability and temperature of incubation (Tab 3).

Samples incubated with 1% CH_4 (v/v) showed the highest activity in the 20-40 cm layer of L location, independently of temperature, whereas MTA decreased in lower layers. The highest MTA characterised the layer of 20-40 cm at 10 and 20°C in T location, while at 5°C the maximal value, above 10 mg CH_4 kg D.W.$^{-1}$ d^{-1}, was observed in the layer of 60-80 cm. A different situation was found in site H. At 5°C MTA increased with depth while at 10 and 20°C it rose only to 40-60 cm and next it declined slightly, but significantly (Tab. 3).

We assumed that the increase of methane availability modified MTA. In the samples from L site MTA decreased with depth when temperature was higher. The highest MTA was noted in layers of 0-20 cm and 40-60 cm for temperatures of 5 and 10°C, respectively. However, when the temperature increased to 20°C the deepest (60-80 cm) part of the peat profile demonstrated the highest MTA. In the material from site T the highest activity was noted in the surface layer (0-20 cm), irrespective of the temperature, while in H it was found in the layer of 40-60 cm.

Peat samples from particular locations differ in their total organic carbon (TOC) content. The highest value was measured in H, while the lowest in L (Fig. 1A). The sampling site determined also the peat ability for methane oxidation. The highest potential, exceeding 50 mg CH_4 kg D.W.$^{-1}$ d^{-1}, characterized the L location (data from depth of 0-80 cm). In T and H sites MTA was at comparable level of 30-35 mg CH_4 kg D.W.$^{-1}$ d^{-1} (Fig. 1B).

Table 3. Methanotrophic activity in investigated peat profiles under selected environmental conditions

Site	Depth (cm)	1% CH$_4$ (v/v)			5% CH$_4$ (v/v)		
		5°C	10°C	20°C	5°C	10C	20°C
L	0-20	7.80a	15.98b	31.83a	42.15c	46.10b	131.33b
	20-40	32.46d	45.51c	86.10d	13.61a	55.49c	118.72a
	40-60	12.65b	13.99b	47.81b	13.00a	74.69d	137.47c
	60-80	17.95c	5.51a	56.11c	25.33b	30.52a	145.10d
T	0-20	5.12b	6.48b	20.10a	33.74c	49.39d	154.04c
	20-40	8.43c	16.28d	51.16c	8.29a	18.87a	63.26a
	40-60	4.08a	7.63c	20.73a	9.7a	25.80c	83.12a
	60-80	10.15d	3.34a	24.96b	15.01b	21.34b	82.4a
H	0-20	1.38a	2.96a	16.1b	4.75a	11.24a	18.25a
	20-40	3.44b	7.41c	9.58a	6.55b	25.28b	68.69b
	40-60	5.40c	23.45d	46.45d	23.33d	90.38d	191.26d
	60-80	5.48c	4.36b	34.16c	13.72c	36.87c	174.63c

- numbers characterising the properties of peat from the one location, followed by the same letter, do not differ significantly (*P*>0.05).

Statistical analysis of all the data confirmed that the raise of temperature stimulated considerably MTA. The most evident increase was observed when the temperature rose from 10 to 20°C; under these conditions MTA was 2.8 times more efficient (Fig. 1C). Statistical analysis of all collected data demonstrated that MTA was significantly modified by substrate availability. Increase of CH$_4$ concentration from 1 to 5% (v/v) induced the increase of MTA from 20 to almost 60 mg CH$_4$ kg D.W.$^{-1}$ d^{-1} (Fig. 1D).

DNA was isolated from the samples characterised by the highest MTA. In general, methane oxidation was the most efficient in the samples incubated at 20°C and in CH$_4$ concentration of 5% (v/v). The most active layers were: 60-80 cm in site L, 0-20 cm in T, and 40-60 cm in H.
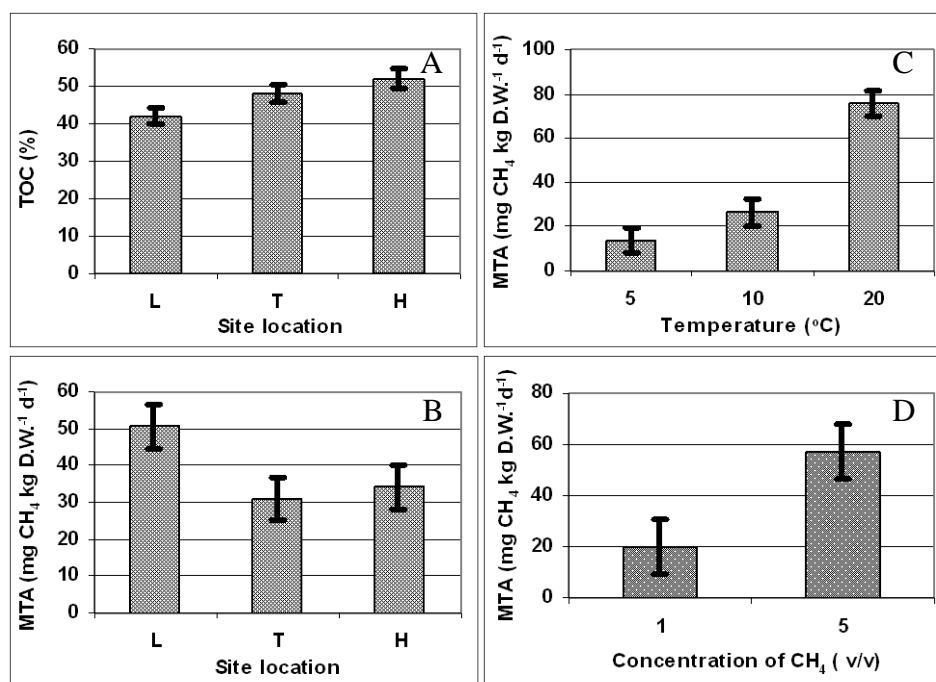
Fig. 1. Total organic carbon (TOC) content in sampling site (A) and the influence of type of sampling site (B), temperature (C) and CH_4 concentrations (D) on methanotrophic activity (MTA), n = 72, error bars indicate the 95% of confidence intervals

Identification of genes specific for methanotrophs

In the case of *pmoA* gene positive results for used pairs of primers A189f/mb661r in material from L were obtained (500 bp), while in the case of 16S rRNA gene and primers Type IIf/Type IIr we got a PCR product (525 bp) in the material from H. No results were found after using primers Type If/Type Ir. In samples from transition moor peat (T) none of the primers used gave products.

DISCUSSION

Methanotrophic activity

The MTA of investigated peatlands from Polesie Lubelskie Region was in a wide range from 1.38 to 191.3 mg CH_4 kg D.W.$^{-1}$ d^{-1}. According to literature data, higher values of MTA were determined in peat bogs (59.2-980.8 mg CH_4 kg D.W.$^{-1}$ d^{-1}) of north-east England (Chen *et al.* 2008) and in soil from trans-arctic climate (17.3-311 mg CH_4 kg D.W.$^{-1}$ d^{-1}) of Siberia (Knoblauch *et al.* 2008).

Comparable MTA data to those obtained in our study were found in acidic forest soils (38.4-169 mg CH_4 kg D.W.$^{-1}$ d^{-1}) from the area of England (Radajewski *et al.* 2002) and the state of Iowa (111.4 mg CH_4 kg D.W.$^{-1}$ d^{-1}) in the U.S. (Chan and Parkin 2001). Most of the wetlands described in literature were characterised by lower values, as in boreal peatlands of Finland (Kettunen *et al.* 1999), Canada (Moore and Dalva 1997), North Carolina (Megonigal and Schlesinger 2002) and some bog and forest soils of Alaska (Whalen and Reeburgh 1996) where maximal MTA level did not exceed 13 mg CH_4 kg D.W.$^{-1}$ d^{-1}.

Obtained sequences of *pmoA* gene fragment demonstrated the highest similarity (99%) to uncultured representatives of the family *Methylococcaceae* which was found in freshwater sediment in a region of Russia and in the range of this family especially to representatives of the genus *Methylobacter* sp. (98%) which was noted in the sediments of lake Washington (USA) (Auman *et al.* 2000). 99-95% similarity was also reported to an uncultured methanotrophic bacterium found in roots of rice, or soils in which rice is practiced (Lüke *et al.* 2010, Qiu *et al.* 2008, Shrestha *et al.* 2008, Qiu *et al.* BLASTN nucleotide databases) and methanotrophic microorganisms (98%) found in methane seep along the North American continental margin (Tavormina *et al.* 2008).

In the case of obtained fragments of 16S rRNA gene from microorganisms inhabiting high moor peatland, 98% of similarity to two species of *Methylosinus* and *Methylocystis* was estimated. Similar *Methylosinus* sp. were revealed in such environments as a dystrophic peatland lake (Neuglobsow, Germany), oxic surface sediment of lake Kinneret (Israel) or soils in Germany (Heyer *et al.* 2002, Knief and Dunfield 2005). Likewise, *Methylocystis* sp. species in lake sediments, Syracuse (USA), wetland soils (Norway, Russia, Germany) were detected (Wartiainen *et al.* 2006, Heyer et al. 2002, Costello and Lidstrom 1999, Galchenko *et al.* 1980). Particular data are presented in Table 4.

Sequencing analysis indicated that in low moor peatland most common are representatives of *γ-Proteobacteria,* exactly Type I, while in high moor peatland *α-Proteobacteria*, that is Type II. This might be caused by pH regime. Earlier works indicated that acidic peat is colonised mainly by methanotrophic representatives of the *α-Proteobacteria: Methylocystis* spp., *Methylocella* spp. and *Methylocapsa*s pp. (Dunfield *et al.*, 2003, Berestovskaya *et al.* 2005, Dedysh 2009). However, species from *Methylobacter* genera are also found in peatlands. It was found that Type I of methanotrophs were more abundant than Type II and members of the genus *Methylobacter* were predominant (Vecherskaya *et al.* 1993, Trotsenko and Khmelenina 2005).

The results should be considered as preliminary and further identification experiments of peatland methanotrophs from Polesie Lubelskie Region are needed.

Table 4. Similarity of received sequences to other methanotrophic bacteria

Fragments of compared sequences	Similar microorganisms	Similarity (%)	Source
	Uncultured *Methylococcaceae* EU722420.1	99	BLASTN nucleotide databases
	Uncultured methanotrophic bacterium FN600075	99	Lüke *et al.* 2010
	Methylobacter sp. AY007286.1	98	Auman *et al.* 2000
pmoA gene from low moor peat (A189f/mb661r)	Uncultured methanotrophic bacterium EU444867	98	Tavormina *et al.* 2008
	Uncultured methanotrophic bacterium, FM986052.1	96	Qiu *et al.* 2008
	Uncultured methanotrophic bacterium AM849714.1	96	Shrestha *et al.* 2008
	Uncultured methanotrophic bacterium FM986052	95	Qiu *et al.*, BLASTN nucleotide databases
	Methylosinus sp. strain L6 AJ868422.1	98	Knief and Dunfield 2005
	Methylosinus sp. strain F10V2a AJ458504.1	98	Heyer *et al.* 2002
	Methylosinus sp. strain KS8a AJ458493.1	98	Heyer *et al.* 2002
16S rRNA from high moor peat (Type IIf/ Type IIr)	*Methylosinus trichosporium,* strain KS24b AJ458496.1	98	Heyer *et al.* 2002
	Methylocystis parvus strain 57, AJ458508.1	98	Costello and Lidstrom 1999
	Methylocystis parvus, strain 81, AJ458476.1	98	Heyer *et al.* 2002, Galchenko *et al.* 1980
	Methylocystis rosea, strain SV97T, AJ414656.1	98	Wartiainen *et al.* 2006
	Methylocystis echinoides, strain IMET 10491, AJ458473.1	98	Heyer *et al.* 2002

CONCLUSIONS

1. In all of the tested peat samples methanotrophic activity (MTA) was found and it ranged from 1.38 to 191.26 mg CH_4 kg D.W.$^{-1}$ d^{-1}.

2. Methanotrophic activity, among tested temperatures, was the highest at 20°C and the maximum values reached were 46-86 (1% CH_4 v/v) and 145-191 (5% CH_4 v/v) mg CH_4 kg D.W.$^{-1}$ d^{-1}. The most active was the peat from low moor peatland.

3. MTA of peat was 3-times higher when the initial concentration of methane was 5% in comparison to the combination with 1% CH_4 (v/v).

4. Fragments of 16S rRNA gene from microorganisms inhabiting site H are similar to methanotrophs from the genera *Methylocystis* and *Methylosinus* (Type II of methanotrophs).

5. Fragments of *pmoA* gene from microorganisms inhabiting peat from L location showed similarity to the family *Methylococcaceae* and the genus *Methylobacter* (Type I of methanotrophs).

REFERENCES

Auman A.J., Stolyar S., Costello A.M., Lidstrom M.E., 2000. Molecular characterization of methanotrophic isolates from freshwater lake sediment. Applied and Environmental Microbiology, 66(12), 5259-5266.

Berestovskaya Yu., Rusanov I.I., Vasil'eva L.V., Pimenov N.V., 2005. The processes of methane production and oxidation in the soils of the Russian Arctic Tundra. Microbiology, Vol. 74 (2), 221-229.

Blast Local Alignment Search Tool, http://www.ncbi.nlm.nih.gov/blast

Chan A.S.K., Parkin T.B., 2001. Methane oxidation and production activity in soils from natural and agricultural ecosystems. Published in Journal of Environmental Quality, 30, 1896-1903.

Chen Y., Dumont M.G., Cébron A., Murrell J.C., 2007. Identification of active methanotrophs in a landfill cover soil through detection of expression of 16S rRNA and functional genes. Environmental Microbiology, 9(11), 2855-2869.

Chen Y., Dumont M.G., McNamara N.P., Chamberlaine P.M., Bodrossy L., Stralis-Pavese, Murrell J.C., 2008. Diversity of the active methanotrophic community in acidic peatlands as assessed by mRNA and SIP-PLFA analyses. Environmental Microbiology, 10(2), 446-459.

Chen Y., Murrell J.C., 2010. The aerobic methane oxidizing bacteria (Methanotrophs) in Timmis K. N. (ed.), Handbook of Hydrocarbon and Lipid Microbiology. DOI 10.1007/978-3-540-77587-4_143,Springer-Verlag Berlin Heidelberg.

Costello A.M., Lidstrom M.E., 1999. Molecular characterization of functional and phylogenetic genes from natural populations of methanotrophs in lake sediments. Applied and Environmental Microbiology, 65(11), 5066-5074.

Dedysh S.N., 2009. Exploring methanotroph diversity in acidic northern wetlands: molecular and cultivation-based studies. Microbiology, 78 (6), 655-669.

Dumont M.G., Pommerenke P., Casper P., Conrad R., 2011. DNA-, rRNA- and mRNA-based stable isotope probing of aerobic methanotrophs in lake sediment. Environmental Microbiology, 13(5), 1153-1167.

Dunfield P.F, Khmelenina V.N, Suzina N. E, Trotsenko Y.A., Dedysh S.N., 2003. Methylocella silvestris sp. nov., a novel methanotroph isolated from an acidic forest cambisol. Int. J. Syst. Evol. Microbiol., 53,

1231-1239.

Dunfield P.F., Dedysh S.N., 2010. Acidic Environments in Timmis K.N. (ed.), Handbook of Hydrocarbon and Lipid Microbiology. DOI 10.1007/978-3-540-77587-4_143, Springer-Verlag Berlin Heidelberg.

Galchenko V.F., Nesterov A.I., Andreev L.V., Trotsenko Y.A., 1980. New species of methanotrophic bacteria *Methylocystis*. Pushchino: USSR Academy of Sciences.

Heyer J., Galchenko V.F., Dunfield P.F., 2002. Molecular phylogeny of type II methaneoxidizing bacteria isolated from various environments. Microbiology, 148, 2831-2846.

Holmes A.J., Owens N.J.P., Murrell J.C., 1996, Molecular analysis of enrichment cultures of marine methane oxidising bacteria. Journal of Experimental Marine Biology and Ecology, 203, 27-38.

Hua S.F., Li S.B., Tan H.D., 2007. Molecular sequencing and analysis of soluble methane monooxygenase gene clusters from methanotroph Methylomonas sp. GYJ3. World J. MicroBiol. Biotechnol., 23, 323-330.

Kettunen A., Kaitala V., Lehtinen A., Lohila A., Alm J., Silvola J., Martikainen P.J., 1999. Methane production and oxidation potentials in relation to water table fluctuations in two boreal mires. Soil Biology and Biochemistry, 31, 1741-1749.

Knief C., Dunfield P.F., 2005. Response and adaptation of different methanotrophic bacteria to low methane mixing ratios. Environmental Microbiology, 79, 1307-1317.

Knoblauch C., Zimmermann U., Blumenberg M., Michaelis W., Pfeiffer E.M., 2008. Methane turnover and temperature response of methane-oxidizing bacteria in permafrost-affected soils of northeast Siberia. Soil Biology and Biochemistry, 40, 3004-3013.

Liebner S., Rublack K., Stuehrmann T., Wagner D., 2009. Diversity of aerobic methanotrophic bacteria in a permafrost active layer soil of the Lena Delta, Siberia. Microbial Ecology, 57(1), 25-35.

Lüke C., Krause S., Cavigiolo S., Greppi D., Lupotto E., Frenzel P., 2010. Biogeography of wetland rice methanotrophs. Environ Microbiol., 12(4), 862-72.

McDonald I.R., Bodrossy L., Chen Y., Murrell J.C., 2008. Molecular ecology techniques for the study of aerobic methanotrophs. Applied and Environmental Microbiology, 74(5), 1305-1315.

Megonigal J.P., Schlesinger W.H. 2002. Methane-limited methanotrophy in tidal freshwater swamps. Global Biogeochemical Cycles, 16(4).

Moore T.R., Dalva M.N 1997. Methane and carbon dioxide exchange potentials of peat soils in aerobic and anaerobic laboratory incubations. Soil Biology and Biochemistry, 29(8), 1157-1164.

Murrell J.C., 2010. The aerobic methane oxidizing bacteria (Methanotrophs) in Timmis K. N. (ed.), Handbook of Hydrocarbon and Lipid Microbiology. DOI 10.1007/978-3-540-77587-4_143, Springer-Verlag Berlin Heidelberg.

Qiu Q., Noll M., Abraham W.R., Lu Y., Conrad R., 2008. Applying stable isotope probing of phospholipid fatty acids and rRNA in a Chinese rice field to study activity and composition of the methanotrophic bacterial communities in situ. The ISME Journal: Multidisciplinary Journal of Microbial Ecology, 2(6), 602-614.

Radajewski S., Webster G., Reay D. S., Morris S. A., Ineson P., Nedwell D. B., Prosser J. I., Murrell J. C., 2002. Identification of active methylotroph populations in an acidic forest soil by stable isotope probing. Microbiology, 148, 2331-2342.

Radwan S. (ed.) 2003. Environmental base for the protection and restoration of peat-water ecosystems in the functional area of the Poleski National Park. Environmental monograph (in Polish). Institute of Agrophysics PAS, Lublin.

Sambrook J., Russell D.W., 2001. Molecular cloning: a laboratory manual. 3rd ed. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, New York.

Shrestha M., Abraham W.R., Shrestha P.M., Noll M., Conrad R., 2008. Activity and composition of methanotrophic bacterial communities in planted rice soil studied by flux measurements, analy-

ses of pmo gene and stable isotope probing of phospholipid fatty acids. Environ. Microbiol., 10(2), 400-412.

Szafranek-Nakonieczna A., Bennicelli R.P., 2010. Ability of peat soil to oxidize methane and effect of temperature and layer deposition. Polish Journal of Environmental Studies, 19(4), 805-810.

Tavormina P.L., Ussler W., Orphan V.J., 2008. Planktonic and sediment-associated aerobic methanotrophs in two seep systems along the North American margin. Appl. Environ. Microbiol., 74 (13), 3985-3995.

Trotsenko J.A., Khmelenina V.N., 2005. Aerobic methanotrophic bacteria of cold ecosystems. FEMS Microbiology Ecology, 53, 15-26.

Vecherskaya M.S., Galchenko V.F., Sokolova E.N., Samarkin V.A., 1993. Activity and species composition of aerobic methanotrophic communities in tundra soils. Curr. Microbiol., 27, 181-184.

Wartiainen I., Hestnes A.G., McDonald I.R., Svenning M.M., 2006. Methylocystis rosea sp. nov., a novel methanotrophic bacterium from Arctic wetland soil. Svalbard, Norway (78 degrees N), International Journal of Systematic and Evolutionary Microbiology, 56(PT 3), 541-547.

Whalen S.C., Reeburgh W.S., 1996. Moisture and temperature sensitivity of CH_4 oxidation in boreal soils. Soil Biology and Biochemistry, no 10/11, 1271-1281.

Wojciechowski I. Szczurowska A., 2002. Peat ecosystems. In: Radwan, S. (ed.). Poleski National Park, Environmental monograph (in Polish), Institute of Agrophysics PAS, Lublin, 161-174.

METANOTROFY ODPOWIEDZIALNE ZA UTLENIANIE METANU W NATURALNYCH TORFOWISKACH POLESIA LUBELSKIEGO

Anna Szafranek-Nakonieczna, Zofia Stępniewska, Agnieszka Wołoszyn, Jakub Ciepielski

Katedra Biochemii i Chemii Środowiska, Katolicki Uniwersytet Lubelski Jana Pawła II
Al. Kraśnicka 102, 20-718 Lublin
e-mail: anna.szafranek@kul.lublin.pl

S t r e s z c z e n i e. Potencjalna aktywność metanotroficzna (MTA) została wyznaczona w warunkach laboratoryjnych, w torfach pochodzących z torfowisk reprezentujących typy: wysokie (H), przejściowe (T) i niskie (L), zlokalizowanych na obszarze Polesia Lubelskiego. Badane torfowiska różniły się między sobą pod względem: pH, zawartości TOC, indeksem von Posta oraz wilgotności. Inkubacje przeprowadzono w następujących warunkach: wilgotność w stanie naturalnym (198-719 %w/w), temperatury: 5, 10 i 20ºC, oraz atmosfera wzbogacona w 1 oraz 5% CH_4 (v/v). Niezależnie od temperatury, niższe wartości MTA (1,38-51,16 mg CH_4 kg $D.W.^{-1}$ d^{-1}) wyznaczono dla torfu inkubowanego w atmosferze wzbogaconej o 1% CH_4(v/v). Na MTA istotny wpływ wykazywała również temperatura oraz lokalizacja punktu poboru prób. Z najaktywniejszych metanotroficznie torfów (20ºC, 5% CH_4 v/v) izolowano całkowite DNA , na którym przeprowadzano reakcje PCR powielające fragment genu *pmoA* (startery A189f/mb661r) oraz sekwencję 16S rRNA (startery Typ If /Typ Ir oraz Typ IIf /Typ IIr), specyficzne dla bakterii metanotroficznych. Pozytywny wynik reakcji PCR ze starterami genu *pmoA* otrzymano dla materiału pochodzącego ze stanowiska L, sekwencjonowanie wskazało na obecność w tym materiale metanotrofów w największym stopniu podobnych do gatunków z rodziny *Methylococcaceae*, natomiast na podstawie sekwencji genu 16S rRNA pochodzącego z mikroorganizmów zasiedlających stanowisko H stwierdzono ich duże podobieństwo do przedstawicieli rodzaju *Methylocystis* i *Methylosinus.*

S ł o w a k l u c z o w e: aktywność metanotroficzna, metanotrofy, torf