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pmoA BASED DETECTION OF METHANOTROPHIC BACTERIA IN COAL-BED ROCKS OF THE LUBLIN COAL BASIN

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A b s t r a c t. Methane is one of the most important greenhouse gases. In spite of its low mixing ratio (1775 ppbv), it is responsible for 20-30% of global warming. However, factors influencing methane fluxes to the atmosphere from different sources are still under debate. One of the most important elements of the methane cycle are methanotrophic bacteria. This unique group of *Proteobacteria* utilises methane at various levels, from atmospheric concentrations to several percentages, using molecular oxygen. Methanotrophs have been found in many terrestrial, aquatic and subsurface environments, there is however little information about methanotrophs connected with coal deposits. In the study, the presence of methanotrophic bacteria in coal–associated rocks of the Lublin Coal Basin (LCB) was confirmed by the methanotrophic activity tests of fresh and autoclaved samples from depths of 914 m and 997 m below the surface. Methanotrophs were also successfully enriched on nitrate minimal salts medium and identified based on the cloned pmoA sequences. It was found that methane-oxidising bacteria present in the LCB are highly similar to *Methylosinus*, *Methylocystis* and *Methylocaldum* species. It was assumed that biological oxidation may be one of the processes influencing methane concentrations in coal-beds and that rocks excavated with coal may serve e.g. as methanotrophically active covers preventing CH_4 emission from landfills.

K e y w o r d s: methanotrophs, methane, coal-bed rocks, *particulate methane monooxygenase*

INTRODUCTION

The process of biological methane oxidation constitutes a critical link in the global carbon cycle and is both ecologically and technologically important.

Methane is the main hydrocarbon present in the atmosphere (with average concentration of 1.775 ppbv in 2005). Despite its short residence time in the atmosphere (about 10 years), methane ability to absorb infrared radiation makes it 20 to 30 times more efficient than CO_2 as a greenhouse gas.

Data show that methane's current mixing ratio is more than twice that in the pre-industrial era and remains in strong correlation with the growth of human population, the development of industry and agriculture. About 60% of overall methane sources is considered to be of anthropogenic origin (coal mining is estimated to contribute about 8% of this) (Bosquet *et al*. 2006, IPCC 2001, 2007).

The major terrestrial CH_4 sink is the aerobic oxidation of methane which is known to be carried by distinct populations of methanotrophic bacteria, possessing enzymes specialised to have a high or low affinity for methane (Hanson and Hanson 1996). The first type (high-affinity, low activity) is observed at atmospheric CH_4 concentration and is ubiquitous in soils. Bacterial populations responsible for this type of CH_4 consumption were only recently identified as belonging to the genera *Methylocystis* (Bull *et al*. 2000) The second type of methanotrophy (low-affinity, high activity) occurs at CH_4 concentrations higher than 40 ppm and is performed by a wide range of methanotrophs (Hanson and Hanson 1996), belonging to either α- or γ-Proteobacteria. It is assumed that the majority of the estimated 400 to 640 Tg of methane (CH_4) produced annually in anoxic environments (Cicerone and Oremland 1988) is oxidised at the anaerobic-aerobic interface by low-affinity methanotrophs, thereby mitigating the global emissions of this greenhouse gas into the atmosphere (Raghoebarsing *et al*. 2005).

The ability of methanotrophs to oxidise methane is due to the possession of the enzyme methane monooxygenase (MMO). There are two distinct forms of this enzyme, the cytoplasmic soluble methane monooxygenase (sMMO), and the membrane-bound particulate methane monooxygenase (pMMO) (Murrell *et al*. 2000), however only the pMMO is found universally in methanotrophs and is therefore used as a functional marker for these organisms (McDonald *et al*. 2008).

Molecular ecology techniques (based on PCR detection of pMMO molecular markers – especially with the use of *pmoA* gene targeted primers) enable detection of methanotrophic bacteria in various aquatic and terrestrial environments worldwide. The majority of methane-consuming bacteria appear to be neutrophylic and mesophylic, however, in the last decade isolation and characterisation of methanotrophs from extreme environments like mud volcanoes, hot springs, permafrost or soda lakes was initiated. Those extremophiles possess adaptations (osmoadaptation, thermoadaptation) that enable them to survive in severe environmental conditions and which can be used for biotechnological purposes. High efficiency of CH_4 incorporation into biomass, wide range of reactions that can be carried by methane monooxygenases (MMOs), ability to synthesise single cell proteins bioprotectans and biopolimers, make methanotrophs a potential tool for biotechnology.

Recently it has been found that methane-oxidising bacteria are present also in carboniferous rocks surrounding seams of the Lublin Coal Basin (LCB). Their presence in coal-surrounding geological strata brings new information about

global C cycling, and helps to understand better the mechanisms influencing coal-bed gas composition and origin. Furthermore, methanotrophic bacteria retrieved from the coal-bed, due to their survival skills, may have an application for biotechnical purposes.

Therefore, the aim of the present study was to determine methanotrophic activity of the rocks originating from different depths of the carboniferous LCB stratum and to identify culture-susceptible methanotrophs with the use of pmoA-targeted assay.

MATERIAL AND METHODS

Sampling site

Carboniferous stratum in the Lublin Coal Basin is located at the depth of c.a. 700 m.b.s (meters below surface). There are c.a. 80 coal seams accompanied mostly by claystones. The thickness of coal bodies ranges from 0.05 to 3.8 m. Most of the coal seams are located in the Upper Carboniferous, Westfalian B strata and are of limnic-fluvial origin (Kotarba 2003).

Rocks were collected from two depths, from the area of seam 382 (S-108) (914 m below surface) and seam 390 (BR-3SP-126) (997 m below surface). Sample S-108 was hammered manually, from the surrounding of a freshly excavated wall, whereas BR-3SP-126 was obtained from the core that was drilled in the bottom of seam 385/5 towards deeper coal layers that will be exploited in the future.

Samples were crushed into pieces and ground in a mill (Testchem, Poland) to 2 mm maximum grain diameter.

Incubations

Incubations, aiming to determine the methanotrophic activity (MA) of the investigated rocks, were commenced within 5 days of collecting the samples.

MA was determined at oxic conditions at a temperature of 30^{o}C. Triplicate samples (15 g) were placed in dark bottles (60 cm^3), filled with deionised water to obtain sample moisture adequate to 100% of water holding capacity, then closed with rubber septa, capped with an aluminium cap and sealed with paraffin. For experiments an initial concentration of ca. 10% (v/v) CH_4 was obtained by replacing an appropriate volume of air with high purity (99.99%) methane (Praxair, Poland) using a gastight syringe (5 ml, SGE, Australia). The headspace concentrations of gases (CH_4, CO_2, O_2) were determined using a gas chromatograph (3800 GC Varian, USA) equipped with flame ionisation (FID) and thermal conductivity (TCD) detectors. Gases were separated on Molecular Sieve 5A, 0.53 mm ID, 30 m length and Poraplot Q, 0.53 mm ID, 25 m length columns

(Varian, USA) using helium as the carrier gas. The analyses were carried out under the following conditions: injector temperature 120°C, oven temperature 40°C, temperature of detectors: 120°C and 200°C for TCD and FID. Incubation times varied depending on the activity of the sample and included 8 to 10 measurement points. Conditions under which the rocks were incubated were previously confirmed as optimum for induction of methanotrophic activity by Stępniewska and co-workers (2004) who found that further increase of substrate concentrations (above 10%) did not result in elevated consumption rates. Water content (100% water holding capacity) in experimental treatments was chosen based on preliminary experiments.

The specific methanotrophic activity (μM CH_4 g $^{-1}$day $^{-1}$) of particular rocks was calculated from the slope of the regression line of the measured CH_4 molar amounts vs. time. In parallel to the fresh rock, incubations of autoclaved (2 h, 121°C, 15 psi) (Hiclave HG-50) samples were performed.

Enrichment cultures

Methanotrophic cultures were established in 10 ml of NMS medium (Whittenbury, 1970) that contained: KNO_3, 1.0; KH_2PO_4, 0.54; $Mg_2SO_4 \cdot 7H_2O$, 0.2; $CaCl_2 \cdot 2H_2O$, 0.015. $CaCl_2$, Mg_2SO_4 (g l^{-1} distilled water at pH 6.8) and trace elements added after autoclaving. The bottles inoculated with coal-bed rocks were incubated under 10% v/v CH_4 in air at 30°C for 10 days.

DNA isolation

DNA extraction from methanotrophs enrichment cultures was performed using the method described by Sambrook and co-workers (1989), with minor changes. Ten days enrichment cultures were harvested by centrifugation and the pellets suspended in TE buffer and subjected to lysis using GES solution (5M *g*uanidine thiocyanate, 100 mM EDTA, 0.5% sarcosyl [pH8]). DNA was purified using ice-cold solution of ammonium acetate (7,5M) and subsequently chloroform : isoamyl (24:1) mixture and cell debris removed by centrifugation. DNA was precipitated at –20°C with isopropanol for two hours, pelleted by centrifugation at 17 500 x g for 30 min, rinsed 5 times with 70% v/v ethanol and resuspended in 30 ml of ultrapure, DNAse free water.

PCR and cloning

Amplifications were performed in TProffessional Gradient System (Biometra, Germany) using primer set designed by Costello and co-workers (1999) for the detection of the pmoA gene of the methanotrophs. The primer sequences were as follows: forward – A189: GGNGACTGGGACTTCTGG and reverse: mb661 –

CCGGMGCAACGTCYTTACC. PCR reactions were carried out in a total volume of 25 ml in 0.2 ml tubes. Each PCR mix consisted of 2 µl DNA matrix, 12.5 µl PCR Master Mix 2x (Fermentas), 1 µl of each primer (10mM) and 8.5 µl nuclease free water (Fermentas). PCR conditions consisted of an initial denaturation step at 94°C for 4 min, followed by 30 cycles at 94°C for 1 min, 54.5°C for 55 s and 72°C for 55 s. The final extension step was at 72°C for 3 min.

PCR products were checked for size and purity on 1% w/v agarose gels. Products of the correct size were purified using QIAquick PCR Purification Kit (Qiagen) and ligated into pGem®-T Easy vector supplied by Promega, according to the manufacturer's instructions. Five random selected clones from each sample were sequenced at Genomed Sp. z.o.o.(Poland) with the use of vector-specific flanking primers (M13f and M13r). Nucleotide-nucleotide Blast (BlastN) was used to search the GeneBank for the nearest relative sequence. Original *pmoA* sequences achieved for the coal-bed methanotrophs and selected public-domain sequences were aligned and the phylogenetic tree was built using the neighbourhood-joining method (Saitou and Nei 1987).

RESULTS AND DISCUSSION

The dynamics of CH_4, O_2 and CO_2 concentrations in gaseous phase of incubations performed with the use of fresh and autoclaved samples revealed that methane uptake in the investigated rocks is due to the activity of methanotrophic bacteria. In the fresh rocks, after c.a. 10 days of lag phase CH_4 was rapidly utilised with the use of molecular oxygen, which was confirmed by the parallel drop in the concentrations of those gases phase and simultaneous growth of CO_2 mixing ratio. Contrastingly, methane concentration in the sterilised samples remained nearly unchanged during the whole incubation period (Fig. 1).

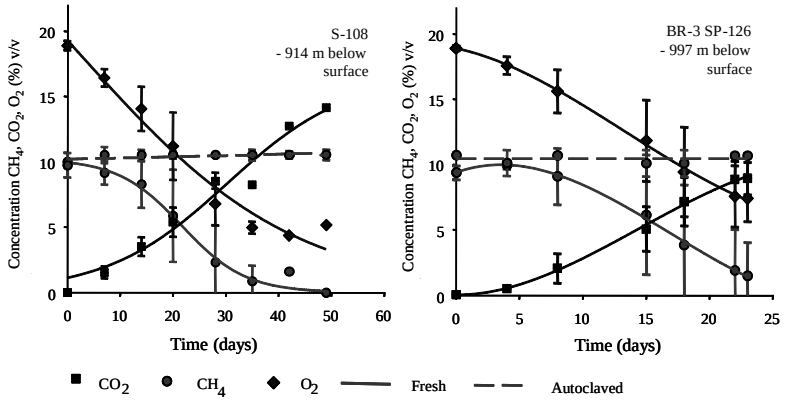

Fig. 1. Concentration dynamics of CH_4, O_2 and CO_2 in incubations of the coal-bed rocks. Data points represent an average value and bars standard deviation of 3 experimental replicates

The MA of the coal-bed rock S-108 was 0.906 and that of BR-3SP-126 was 1.496 μMCH_4 g^{-1} day^{-1}, respectively. The values obtained for MA are comparable to methanotrophic activities found in surface environments, e.g. in peat – 1.656 μMCH_4 $g^{-1}day^{-1}$ (Freeman *et al.* 2002) or lake sediment – 0.11 μMCH_4 $g^{-1}day^{-1}$. Also noteworthy is the fact that methane uptake by bacteria present in the investigated rocks began within just c.a. 5 days from the beginning of incubation at optimal conditions.

Methanotrophic bacteria in the coal-bed were able to survive in the deep subsurface conditions where high lithostatic pressure (Cała *et al.* 2004), low concentration of oxygen (Kotarba 2001) and arid conditions prevail. The mechanisms of methanotrophs survival in coal-bed must have been successful enough to support bacterial life for extended periods of time as geological settings and impermeability of the carboniferous formations in the LCB area imply that they were isolated from water and nutrient supply since the Pleistocene (2 Ma) (Kotarba and Clayton 2003).

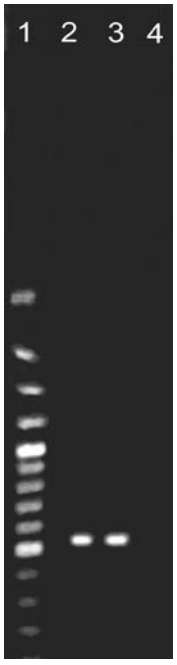

Fig. 2. Electropherogram of PCR products amplified with 189f/mb661r primers set. 1 – GeneRuler™ Ladder Plus, 2 – S-108, 3 – BR-3SP-126, 4 – negative control

Interestingly, higher MA was found in rock BR-3 SP-126, retrieved from the layer of the carboniferous stratum lying deeper (997 m below surface) than in rock S-108 (914 m below surface), which suggests that the methanotrophs are not sensitive to shifts in lithostatic pressure (which, based on the average gradient of 23 kPa m^{-1} in the continental crust (Gentzis 2009) was calculated to be c.a. 23 MPa).

Identification of the microorganisms potentially applicable in biotechnology was performed based on the DNA, successfully isolated from methanotrophic enrichment cultures of both investigated coal-bed rocks. PCR amplification with *pmoA*-targeted primers generated products of proper size (c.a. 510 bp) (Fig. 2.) which were subsequently ligated into pGem®-T Easy vector, cloned and sequenced.

It was found that *pmoA* sequences achieved from rock S-108 were closely related to representatives of *Methylocaldum*, *Methylosinus* and *Methylocystis*, whereas those from rock BR-3 SP-126 only to the latter gender (Fig. 3). The sequences of *Methylocaldum* (92-97% identity) represented *M. gracile* [U89301.1] (Bodrossy *et al.* 1997), and uncultured bacteria isolated from landfill cover soils operating at different climatic conditions e.g. in Austria [AY195664.1] (Bodrossy *et al.* 2003), Taiwan [EU275141.1] or Canada [EU647271.1].

Sequences of *pmoA* from S-108 enrichment culture were also highly similar to *Methylosinus trichosporium*, including strains M23 isolated from mangrove roots in the western India

[AJ459037.1] (97-99%), O19/1 isolated from Baltic sediments [AJ459021.1] (94-95%) and KS18 originating from the aerated lake sediments [AJ459032.1] (95%).

Sequences obtained from both rocks showed 97-100% similarity to *Methylocystis* isolated from experimental lysimeters [e.g.GQ857552.1, GQ857557.1, GQ857560.1, GQ857561.1, GQ857564.1, GQ857572.1].

The results obtained show that cultivation-susceptible methanotrophic community of the LCB coal-bed rocks consists mostly of bacteria capable of resting stage formation, which is a logical explanation of their presence in the severe, moisture and oxygen-depleted conditions of the coal-bed. Other authors investigating deep subsurface environments also found similar structures of the methanotrophic community. However, so far the majority of studies concerned aquatic habitats, e.g. Kotelnikova and Pedersen (1996) found *Methylosinus* and *Methylocystis* species at the depth of 400 m, in igneous granitic rock aquifer at Äspö (Sweden), and representatives of the same genera were enriched from groundwater sandy aquifiers in U.S. by Bowman and co-workers (1993).

Furthermore, in spite of the fact that their present-day activity is inhibited by low oxygen levels in the coal-bed gas, it cannot be excluded that methanotrophic activity in the past ages might have brought down methane concentrations in the coal-bed to present-day low values. Moreover, the lack of oxygen might have been one of the factors enhancing methanotrophs survival in unfavourable conditions of the coal-bed. It was described by Roslev and co-workers (1995) that the recovery of MA by CH_4-starved soils and sediments was better in samples starved in anoxic conditions rather than oxic. The authors attributed a better survival of methanotrophs in anoxic conditions to the destructive force coming from free oxygen radicals in starved methanotroph under ambient O_2.

Another mechanism that might have supported the survival of methanotrophs in oxygen-depleted environments might have been the synthesis of poly-3-hydroxy butyrate (PHB) – an internal storage polymer which is used by some methanotrophs as an alternative reducing-energy source under starvation conditions. The *pmoA* sequences achieved from the enrichment cultures indicate that there is a close relationship between methanotrophic bacteria found in the coal-bed rocks with species having well documented ability to synthesise PHB like Methylosinus trichosporium OB3 (Doronina *et al.* 2007). PHB is currently one of the most promising biopolymers (Bonartsev *et al.* 2007), therefore further research should be undertaken to verify whether strains present in the coal-bed could be used for commercial PHB production.

The presence of spore- and cyst-forming methanotrophs (*Methylocystis* and *Methylosinus*) may also enable application of the coal-bed rocks as methane–oxidising landfill covers. High efficiency for methane removal by microbiota inhabiting the investigated rocks can be compared to other materials supporting

the growth of methanotrophic bacteria and already being used in methane biofiltration (Stępniewska *et al.* 2010). The extreme survival skill of the methanotrophic bacteria in arid conditions of the coal-bed may be important, as moisture of the landfill covers is highly variable, especially at the beginning of reclamation, when there is no plant cover.

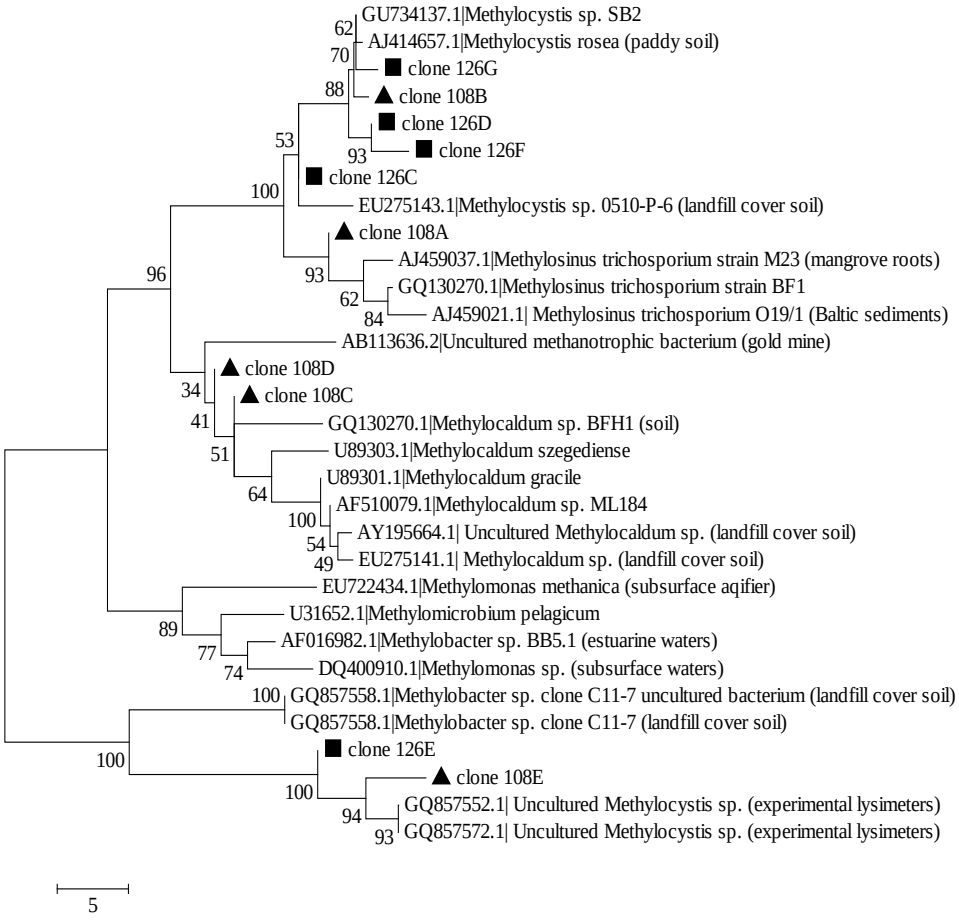

Fig. 3. Phylogenetic tree of bacterial amplicons. Nucleotide sequences were aligned with Clustal W and phylogenetic trees were created with MEGA 5.1 software using the neighbour-joining method (Saitou and Nei 1987); the significance of the junctions was established using the bootstrap method (1000 replicates) (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Nei-Gojobori method (Nei and Gojobori 1986) and are in the units of the number of synonymous differences per sequence

CONCLUSIONS

1. Coal-bed rock surrounding seams 382 and 390 in the Lublin Coal Basin are habitats of methanotrophic bacteria.
2. Methanotrophic activity of the coal-bed rocks measured at 10% CH_4, 100% WHC and 30°C in samples collected from the depths of 914 and 997 m below surface was 0.906 and 1.496 $\mu MCH_4\ g^{-1}day^{-1}$, respectively.
3. Biological methane oxidation might have been the reason for present-day low methane levels in the LCB formations.
4. Methanotrophic bacteria, revealing high similarity of *pmoA* sequences to the genera of *Methylocistis*, *Methylosinus* and *Methylocaldum*, can be enriched from the coal-bed.
5. High survival skills of the coal-bed methanotrophs may enable application of the carboniferous rocks as landfill cover soils.
6. Enrichment cultures built on the basis of the coal-bed rocks should be subjected to further research aiming to determine other biotechnological applications of the isolated methanotrophic bacteria, e.g. PHB synthesis.

REFERENCES

Bodrossy L., Holmes E., Holmes A., Kovacs K., Murrell J., 1997. Analysis of 16S rRNA and methane monooxygenase gene sequences reveals a novel group of thermotolerant and thermophilic methanotrophs *Methylocaldum* gen. nov.. Archives of Microbiology, 168, 493-503.

Bodrossy L., Stralis-Pavese N., Murrell J., Radajewski S., Weilharter A., Sessitsch A., 2003. Development and validation of a diagnostic microbial microarray for methanotrophs. Environmental Microbiology, 5(7), 566-582.

Bonartsev A., Myshkina V., Nikolaeva D., Furina E., Makhina T., Livshits V., Boskhomdzhiev A., Ivanov E., Iordanskii A., Bonartseva G., 2007. Biosynthesis, biodegradation, and application of poly 3-hydroxybutyrate and its copolymers - natural polyesters produced by diazotrophic bacteria. Communicating Current Research and Educational Topics and Trends in Applied Microbiology,Méndez-Vilas A. Ed., 295-307.

Bosquet P., 2006. Contribution of anthropogenic and natural sources to atmospheric variability. Nature, 443, 439-443.

Bowman J., Jimenez L., Rosario I., Hazen T., Sayler, G., 1993. Characterization of the methanotrophic bacterial community present in a trichloroethylene-contaminated subsurface groundwater site. Appl. Environ. Microbiol., 59(8), 2380-2387.

Bull I., Parakh N., Hall G., Ineson P., Evershed R., 2000. Detection and classification of atmospheric methane oxidizing bacteria in soil. Nature, 405, 175-178.

Cała M., Piechota S., Tajduś A,. 2004. State of stress in the ground surrounded by fields in the longwall mining "Bogdanka" Coal Mine. Mining News 55, 46-53. (in Polish)

Cicerone R. J., Oremland R., 1988. Biogeochemical aspects of atmospheric methane. Global Biogeochemical Cycles 2, 299-327.

Costello A., Lidstrom M., 1999. Molecular characterization of functional and phylogenetic genes from natural populations of methanotrophs in lake sediments. Applied Environmental Micro-biology, 65, 5066-5074.

Doronina N., Ezhov V., Trotsenko Y., 2008. Growth of Methylosinus trichosporium OB3b on methane and poly-β-hydroxybutyrate biosynthesis. Applied Biochemistry and Microbiology, 44(2), 182-185.

Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution, 39, 783-791.

Freeman C., Nevison G., Kang H., Hughes S., Reynolds B., Hudson J., 2002. Contrasted effects of simulated drought on the production and oxidation of methane in a mid-Wales wetland. Soil Biology and Biochemistry, 34, 61-67.

Gentzis T., 2009. Stability analysis of a horizontal coalbed methane well in the Rocky Mountain Front Ranges of southeast British Columbia Canada. International Journal of Coal Geology, 77, 328-337.

Hanson R., Hanson T., 1996. Methanotrophic bacteria. Microbiological Review,s 60**,** 439-471

IPCC Climate Change, 2007. The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change.

IPCC Climate Change, 2001. The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change. In, Houghton J.T., Y. Ding, D.J. Griggs, M. Noguer, P.J. van der Linden, X. Dai, K. Maskell, C.A. Johnson eds. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Kotarba M., 2001. Composition of coalbed gases in the Upper Silesian and Lublin Basins Poland. Organic Geochemistry, 32, 163-180.

Kotarba M., Clayton J., 2003. A stable carbon isotope and biological marker study of Polish bituminous coals and carbonaceous shales. International Journal of Coal Geology, 55, 73- 94.

McDonald I., Bodrossy L., Chen Y., Murrell J., 2008. Molecular Ecology Techniques for the Study of Aerobic Methanotrophs. Applied And Environmental Microbiology, 74, 5, 1305-1315.

Murrell J., Gilbert C., McDonald I., 2000. Molecular biology and regulation of methane monooxygenase. Arch. Microbiol., 173, 325-332.

Nei M., Gojobori T., 1986. Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. Molecular Biology and Evolution, 3, 418-426.

Raghoebarsing A.A., Smolders A.J.P., Schmid M.C., Rijpstra W.I.C., Wolters-Arts M., Derksen J., Jetten M.S.M., Schouten S., Damste J.S.S., Lamers L.P.M., Roelofs J.G.M., Op den Camp H.J.M., Strous M., 2005. Methanotrophic symbionts provide carbon for photosynthesis in peat bogs. Nature, 436, 1153-1156.

Roslev P., King G., 1995. Aerobic and Anaerobic Starvation Metabolism in Methanotrophic Bacteria. Applied and Environmental Microbiology, 61, 1563–1570.

Saitou N., Nei M., 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4, 406-425.

Sambrook J., Fritsch E.F., Maniatis T., 1989. A Laboratory Manual in Molecular Cloning. Cold Spring Harbor Laboratory Press, NY, Vol. 1, 2, 3.

Stępniewska Z., Pytlak A,. Wołoszyn A., 2010. Perspectives for methane biofiltration with the use of coalbed rocks originating from ,,Lubelski Węgiel ,,Bogdanka" SA coal mine, Unconventional energy and waste management. Monograph. Wiatr I., Marczak H. red. Lublin. Polish Society of Ecological Engineering, 189-197.

Stępniewska Z., Szmagara A., Stefaniak E., 2004. Optimization of environmental parameters conductive to methane oxidation with coal mine dump rock. Environment Protection Engineering 30, 133-137.

Whittenbury R., Phillips K., Wilkinson J., 1970. Enrichment isolation and some properties of methane-utilizing bacteria. Journal of General Microbiology, 61, 205-218.

DETEKCJA BAKTERII METANOTROFICZNYCH W SKAŁACH PRZYWĘGLOWYCH LUBELSKIEGO ZAGŁĘBIA WĘGLOWEGO W OPARCIU O GEN *pmoA*

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S t r e s z c z e n i e . Metan jest jednym z najważniejszych gazów szklarniowych, który pomimo niskiego stężenia w atmosferze (1775 ppbv) odpowiedzialny jest za 20-30% obserwowanego efektu szklarniowego. W globalnym bilansie CH_4 ważną rolę odgrywają bakterie metanotroficzne. Stanowią one wyjątkową grupę *Proteobacteria* zdolnych do wykorzystania metanu zarówno, gdy jego stężenie jest bardzo niskie (na poziomie atmosferycznym) jak również wysokie, wynoszące nawet kilkadziesiąt procent. Występowanie metanotrofów potwierdzono w wielu lądowych, wodnych i podziemnych ekosystemach, natomiast wciąż niewiele wiadomo na temat mikroorganizmów utleniających metan w otoczeniu pokładów węgla. W bieżącej pracy potwierdzono występowanie bakterii metanotroficznych w skałach Lubelskiego Zagłębia Węglowego (LZW). Biologiczne utlenianie metanu stwierdzono w skałach pochodzących z głębokości –914 m oraz –997 m pod powierzchnią gruntu. Występujące w tych skałach bakterie metanotroficzne zostały namnożone na pożywce NMS i poddane identyfikacji w oparciu o sekwencje genu *pmoA*. Uzyskane sekwencje wykazywały wysokie podobieństwo do gatunków z rodzajów *Methylosinus*, *Methylocystis* and *Methylocaldum*. Uzyskane wyniki wskazują, że biologiczne utlenianie metanu może wpływać na stężenie metanu w wyrobiskach kopalni węgla kamiennego a także stwarzają perspektywę zastosowania skał przywęglowych, jako warstw zabezpieczających przed emisją CH_4 ze składowisk odpadów komunalnych.

S ł o w a k l u c z o w e : metanotrofy, metan, skały przywęglowe, *pmoA*