



Environmental requirements and group-specific identification of methanotrophic bacteria inhabiting, hard coal-associated rocks

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Methane is an atmospheric trace gas about 23 times more effective in trapping IR radiation than CO₂ and contributing in about 20% to global warming. Global atmospheric concentration of methane has more than doubled since the pre-industrial era. The imbalance in methane global budget is a result of anthropogenic emission among which coal mining industry plays predominant role (Nagy, 2006).

Microbial methane oxidation is a biogeochemical process which is important on global scale (IPCC, 2007). Thanks to the variety of adaptations, microbial, methane-oxidizing consortia not only reduce atmospheric CH₄ levels via high affinity metabolism but also prevent the huge amounts of that potent greenhouse gas produced in anoxic environments from leaking into the atmosphere. Methanotrophic bacteria were extensively studied in various environments including upland soils (Bodelier, 2004), rice paddies (Hortz, 2001), wetlands (Raghoebarsing, 2005) as well as a range of aquatic environments (Boetius, 2000; Waekham, 2003). Recent findings revealed that methanotrophs are also well adapted to extreme conditions such as high or low temperature, pH and salinity (Trotsenko, 2002; 2005) whereas scarcely no information on methanotrophs living in coal surrounding sediments is available.

We present results of the methanotrophic activity and identification of methanotrophic bacteria stated in sedimentary rocks surrounding coal deposit located at Lublin Coal Basin (Eastern Poland).

Rock samples were excavated from currently exploited parts of the deposit (seam no. 382, depth 705 m below sea level) and were representative for the bottom, roof (aleuritic claystones) and the parting of the seam (coal shale). Environmental requirements of methanotrophs and the presence of nutrients and metals essential for functioning enzymes involved in methane oxidation reaction chain were examined.

Initial incubations performed at 10% v/v CH₄ and 30 °C revealed methane oxidation in all investigated samples (0.556 – 0.906 $\mu\text{M CH}_4\text{g}^{-1} \text{ day}^{-1}$) and were the highest in bottom rocks. Further incubations of the bottom rocks at 5, 10, 20 and 30°C, moisture contents equivalent to 25, 50 100 and 200% total water capacity and CH₄ concentrations (1, 5, 10, 20 and 30% v/v) were performed. It was found that methanotrophs inhabiting the studied rocks were not capable of oxidizing methane at temperatures below 20 °C. The optimum moisture content for methanotrophs was 100% of the total water capacity of the rock and 20 % v/v CH₄.

Identification of methanotrophic bacteria in enrichment cultures was performed using molecular biology methods. Fluorescence *in situ* hybridization with probes Mg705 (5'fluoresceine), Mg84 (5'Cy3) and Ma450 (5'Cy5) revealed the presence of both type I and II of methanotrophs, which was confirmed by further identification performed by PCR. Amplifications with 16S rRNA targeted, group specific primers confirmed the presence of bacteria belonging to *Methylosinus*, *Methylobacter* and *Methylocystis*.

The results show that coalbed rock are new poorly recognized habitat of methanotrophic bacteria, which so far has not been considered as taking part in methane transformations in coal deposits. The comparison of coalbed rocks from different mines and mining areas and methane in-situ contents with methanotrophic activities are postulated.