



Characterization of methanotrophic bacterial populations in natural and agricultural aerobic soils of the European Russia

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Atmospheric methane contributes to about 20% of the total radiative forcing by long-lived greenhouse gases, and microbial methane oxidation in upland soils is the only biological sink of methane. Microbial methane oxidation in aerated upland soils is estimated as 15 - 45 Tg yr⁻¹ or 3–9% of the annual sink. Therefore there is need of extensive research to characterize methanotrophic activity in various ecosystems for possible application to reduce atmospheric methane fluxes and to minimize global climate change. The vast majority of known aerobic methanotrophs belongs to the *Proteobacteria* and placed in the families *Methylococcaceae* in the *Gammaproteobacteria*, and *Methylocystaceae* and *Beijerinckiaceae* in the *Alphaproteobacteria*. Known exceptions include the phylum *Verrucomicrobia* and uncultured methanotrophs such as *Candidatus 'Methylomirabilis oxyfera'* affiliated with the 'NC10' phylum. Plenty of studies of aerobic methane oxidation and key players of the process have been performed on various types of soils, and it was found that *Methylocystis spp* and uncultivated methanotrophs are abundant in upland soils. Two of the uncultured groups are upland soil cluster alphaproteobacteria (USCa) and gammaproteobacteria (USCg), as revealed by cultivation-independent surveys of *pmoA* diversity.

Russia is extremely rich in soil types due to its vast territories, and most of these soils have never been investigated from the aspect of methanotrophy. This study addresses methane oxidation activity and diversity of aerobic methanotrophic bacteria in eight types of natural aerobic soils, four of which also had been under agricultural use. Methane fluxes have been measured by in situ static chamber method and methane oxidation rates in soil samples - by radioisotope tracer (¹⁴CH₄) technique. Changes in methanotroph diversity and abundance were assessed by cloning and Sanger sequencing, and quantitative real-time PCR of *pmoA* genes.

Methanotrophic population of unmanaged soils turned out to be much low diverse and dominated by uncultivated methanotrophs.. In Podzoluvisol, Luvisol and Meadow Kastanozem we have identified deeply-branching *pmoA* sequences of *Alphaproteobacteria*, only distantly related to *Crenothrix polyspora*, and formed a monophyletic cluster with uncultured methanotrophs from Hawaiian forest soil, soils in Greenland and Cluster I from arctic tundra soils, referred as UNSC (uncultivated natural soil cluster). A new *pmoA* gene-based PCR primer set was designed for detection of UNSC methanotrophs, and the copy numbers in Podzoluvisol was found to be 8.6 × 10⁵ copies g⁻¹ of soil sampled in September 2013. We observed a pronounced shift to cultured methanotrophs with high similarity to *Methylosinus*, *Methylocystis*, *Methylomicrobium*, *Methylobacter*, and *Methylocaldum* in the same soils after agricultural loading. Soils from agricultural sites had larger diversity of methanotrophs, but they failed to make a significant contribution to elimination of methane as observed in both *in situ* and laboratory experiments.

In summary, our study demonstrated that uncultured methanotrophs with *pmoA* monoxygenase distantly related to and *Crenothrix polyspora* and cluster I methanotrophs dominated in methane-oxidizing bacterial communities in unmanaged soils. Thereby, our results highlight the necessity for further studies to be addressed at studying of this group.

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