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Cultivation and molecular analyses of methanotrophic enrichments from Siberian permafrost-affected soils

The Arctic plays a key role in Earth's climate system as global warming is predicted to be most pronounced at high latitudes. Thawing of permafrost could release large quantities of greenhouse gases into the atmosphere, thus further increasing global warming and transforming the Arctic tundra ecosystems from a carbon sink to a carbon source. Therefore, an understanding of the aerobic methane oxidizing community, as the major sink for methane in permafrost environments, is of particular interest.

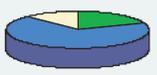
Methods:

Active layer samples from different horizons were taken on Samoylov Island (Lena Delta, Siberia) for cultivation and further molecular characterization of the methane oxidizing bacteria (MOB).

Cultivation was conducted in mineral-salt-media with methane as the sole carbon source at 10 and 28 °C. Amplification of bacterial 16S rRNA genes was carried out using the universal bacterial primers 27F and 1492R, followed by clone library analyses.

Depth [cm]	Temperature [°C]	Copper	Methane [ppm]
9-10	28	+	25.000
16-17	28	+	25.000
30-32	28	+	25.000
9-10	10	+	25.000
16-17	10	+	25.000
30-32	10	+	25.000

Results:

Temp. [°C]	Depth [cm]	Enriched Bacteria [%]	Temp. [°C]	Depth [cm]	Enriched Bacteria [%]
10	9-10		28	9-10	
10	16-17		28	16-17	
10	30-32		28	30-32	

green - α -Proteobacteria; purple - γ -Proteobacteria; blue - β -Proteobacteria; beige - Bacteroidetes, not classified; red - Bacteria, not classified

Conclusion:

In addition to MOB, heterotrophic microorganisms were detected that belong to the phyla Bacteroidetes and Proteobacteria. Clone library analyses showed that cultures incubated at 28°C contained regularly *Methylocella tundrae*. In contrast, cultures grown at 10°C were of higher methanotrophic variety with members related to *Methylobacter psychrophilus* and *Methylobacter tundripaludum*, whose presence in permafrost-affected soils was confirmed by former clone library analyses of environmental DNA (Liebner et al., 2009)³.

In our work we show the considerable impact of cultivation methods on the composition of the enriched bacteria. A shift of temperature in arctic permafrost soils could lead to a change in bacterial community, including methane oxidizing bacteria, and thus influence the global methane cycle.

³Liebner et al., 2009; Diversity of Aerobic Methanotrophic Bacteria in a Permafrost Active Layer Soil of the Lena Delta, Siberia; *Microb Ecol*(57)

Study site:

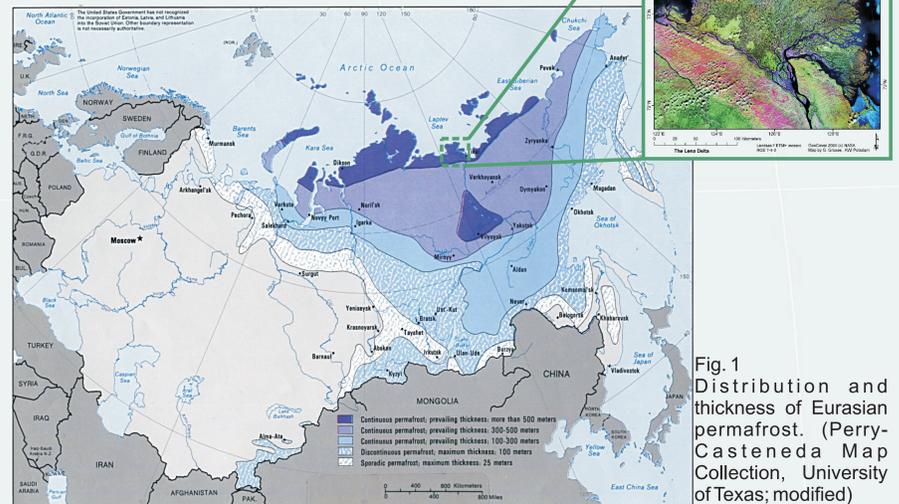
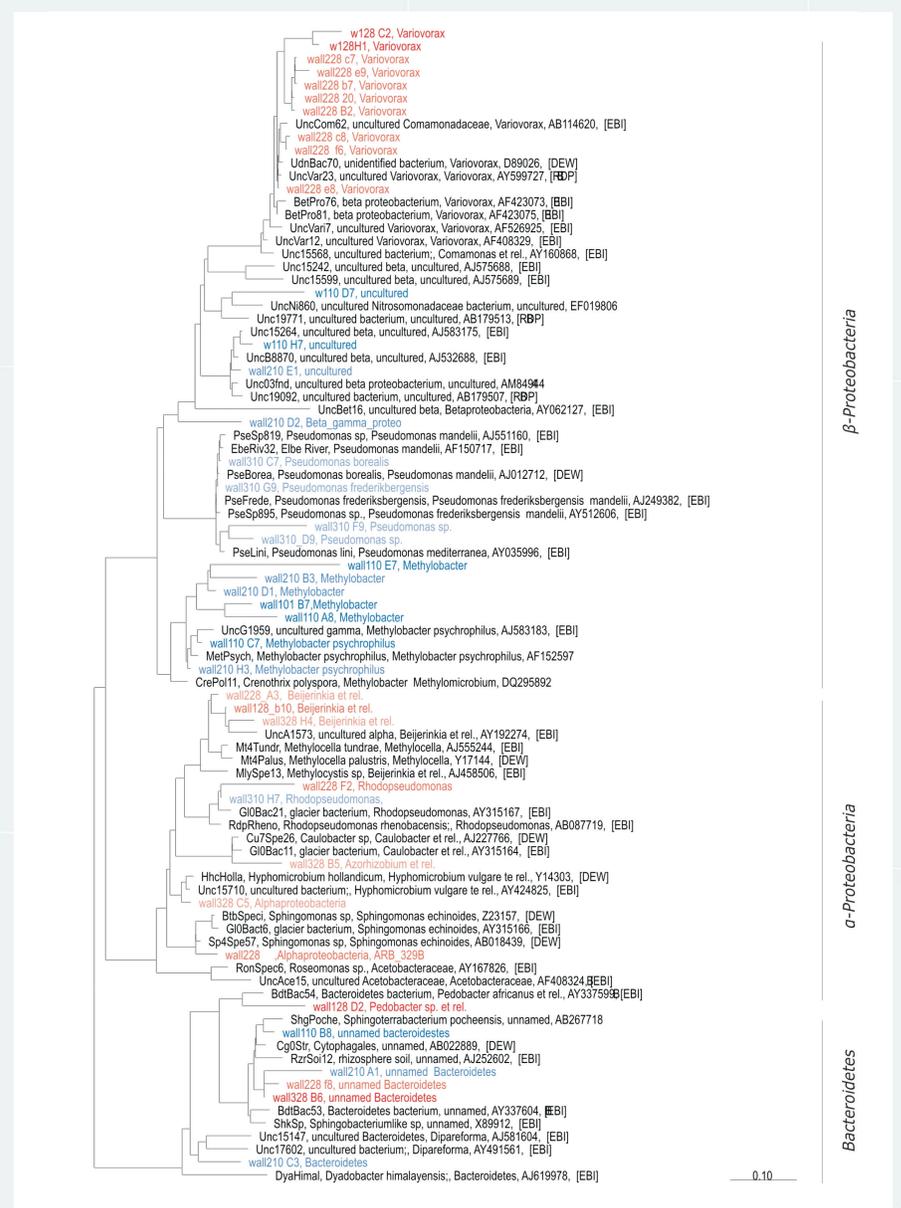


Fig.1 Distribution and thickness of Eurasian permafrost. (Perry-Casteneda Map Collection, University of Texas; modified)

Active layer cores of a low-centered polygon were taken in the scope of the expedition 'Lena 2005'.



β -Proteobacteria

α -Proteobacteria

Bacteroidetes