Northern *Sphagnum* wetlands of Russia:

analysis of biodiversity and functions of microbial assemblages involved in C-cycling

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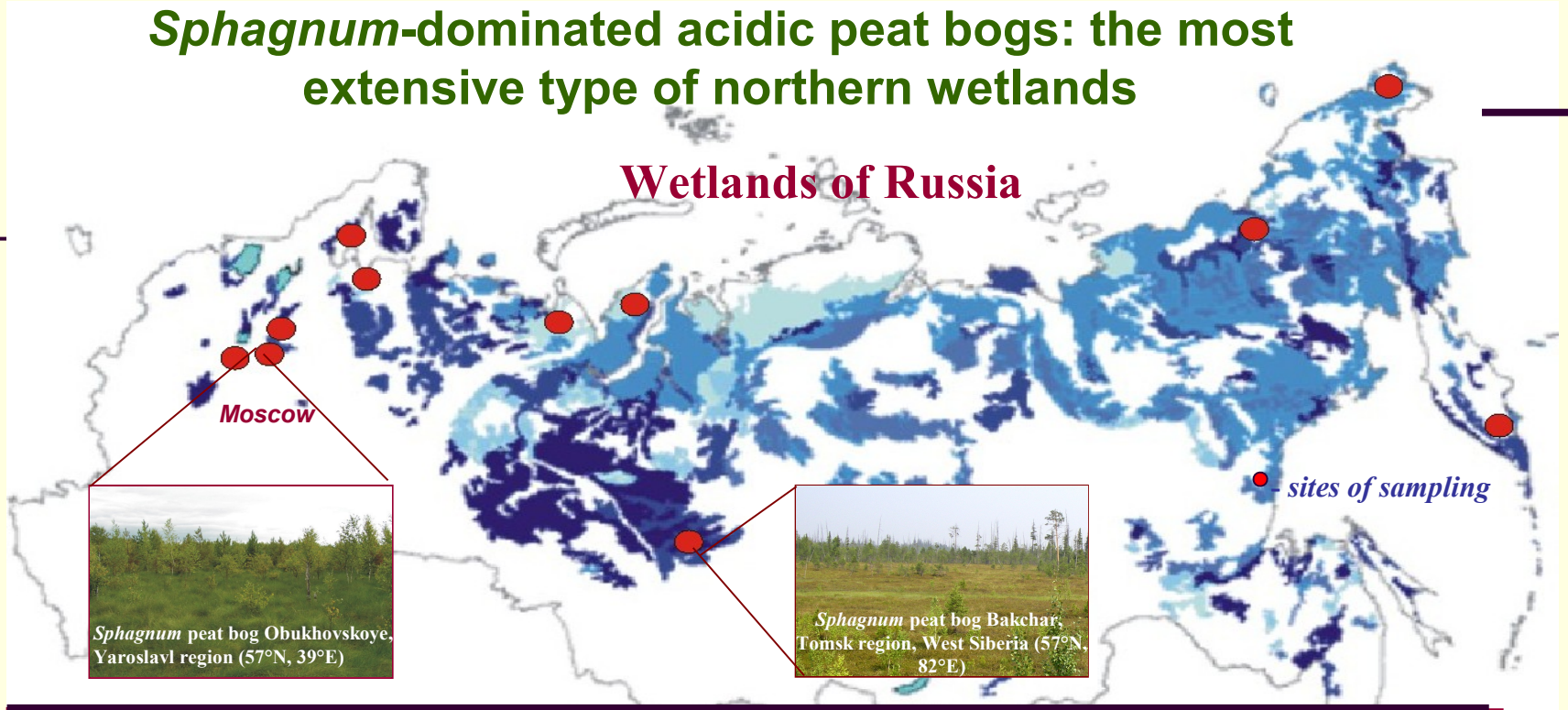
Sphagnum-dominated acidic peat bogs: the most extensive type of northern wetlands



<u>*Sphagnum*-dominated acidic wetlands</u> represent one of the most extensive types of boreal ecosystems in the northern Hemisphere.
Total area of wetlands in the world - 5.3-5.7·1012 м2 (*Aselmann, Crutzen, 1989*; *Fung et al., 1991*). Area of wetlands in Russia - 1.6 ·1012 м2 (*Land resources of Russia, 2002*).

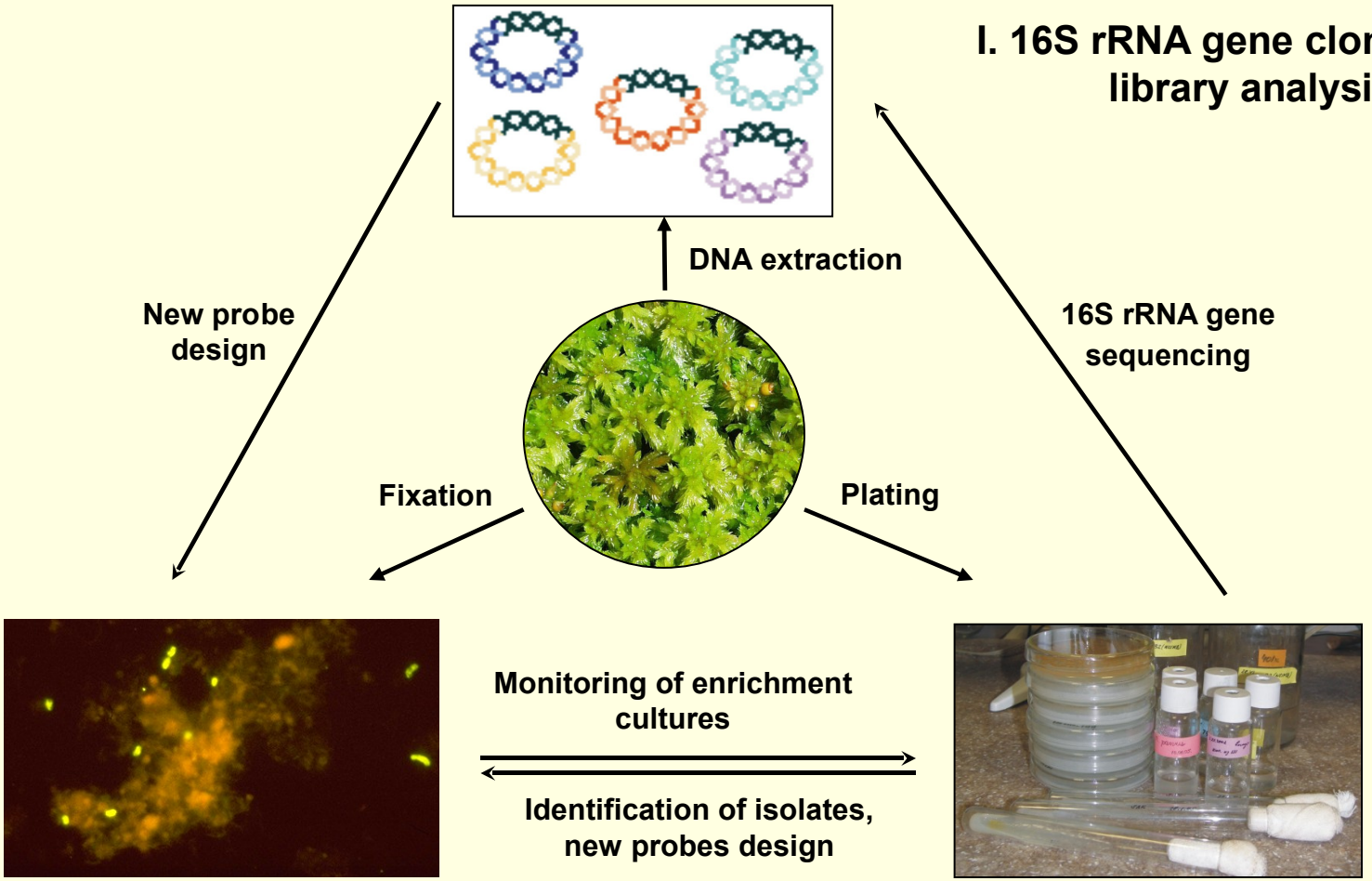
<u>Specific features of *Sphagnum* bog as a microbial habitat:</u>

- ✓high acidity (pH 3.5-5.0)
- ✓low content of mineral salts (5-50 mg/l)
- ✓low buffering capacity
- ✓plant toxic substances
- ✓low temperatures (- 20°C - +20° C)
- ✓freeze-thaw events

<u>The biospheric significance of northern peatlands:</u>

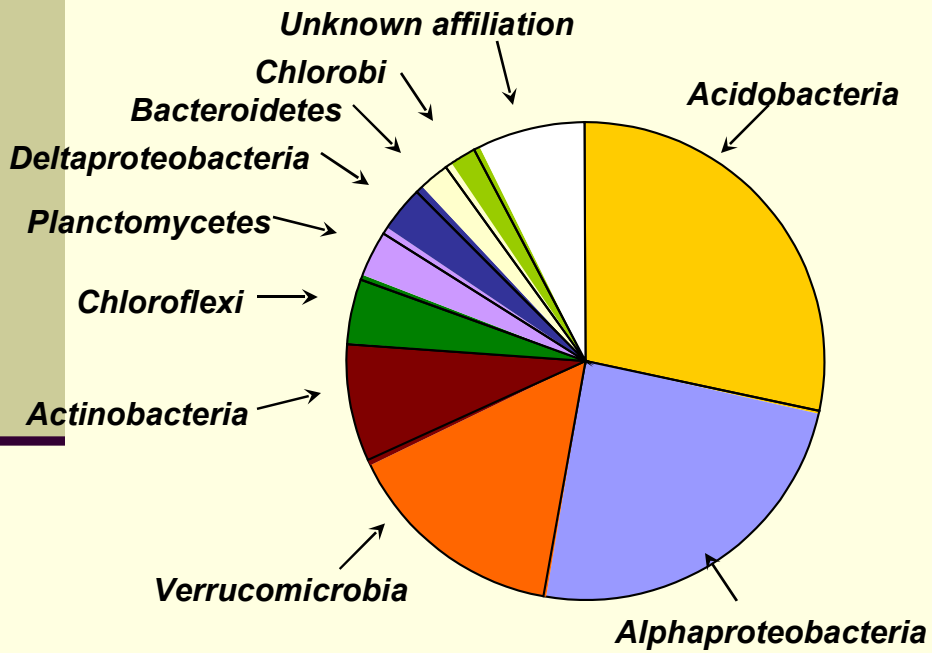
- ✓ important sources of methane and carbon dioxide, the gases that contributes to global warming;
- ✓a major global carbon store harboring approximately 30% of the global reserves of soil organic carbon;
- ✓wetlands determine the hydrology of northern rivers and represent one of the largest reservoirs of fresh water.

Analysis of the *Bacteria* community composition in *Sphagnum* peat (research strategy)



16S rRNA gene clone library data versus FISH analysis

Clone library: The ***Acidobacteria*** and the ***Alphaproteobacteria*** are the most diverse and abundant bacterial groups



Dedysh et al., AEM, 2006, 72: 2110-2117.

FISH analysis: The most abundant bacterial groups are the ***Alphaproteobacteria*** , ***Planctomycetes***, and ***Acidobacteria***.

Oligonucleotide probes:

ALF1b+ALF968 (*Manz et al., 1992*) – specific for the *Alphaproteobacteria*,

HoAc1402 (*Juretschko et al., 2002*) – specific for the *Acidobacteria*,

PLA46+PLA886 (*Neef et al., 1998*) – specific for the *Planctomycetes*.

Population sizes determined by FISH:

Alphaproteobacteria – 1.2 –10.0x10^7 cells g^{-1}

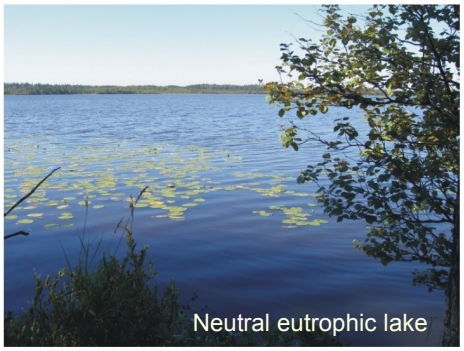
Planctomycetes – 0.4– 2.0 x10^7 cells g^{-1}

Acidobacteria – 0.1– 1.2 x10^7 cells g^{-1} wet peat

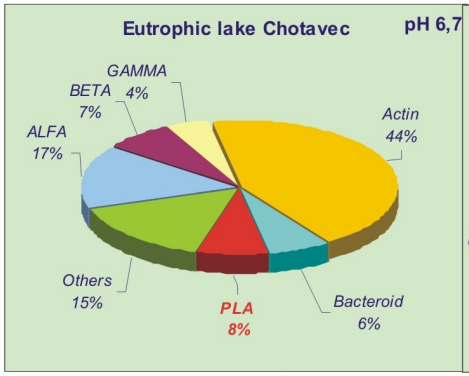
Phylogenetic diversity of bacteria in the water of humic lakes and acidic peat bog of the catchment area.

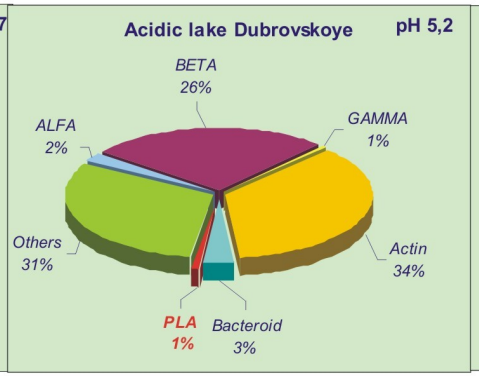
Darwin State Reserve (58° N, 38° E), northwestern Russia.

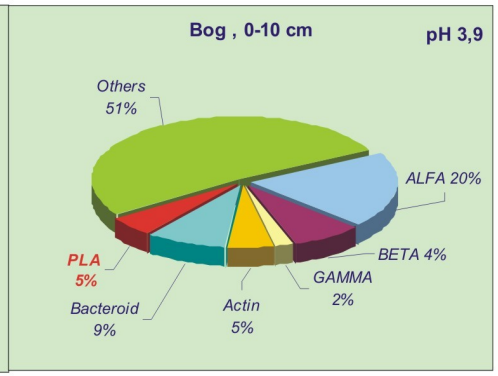
Owing to the rapidly increasing importance of the sustainable management of freshwater resources, detailed knowledge of the diversity, functions and ecology of microorganisms inhabiting the freshwater ecosystems is urgently needed.



FISH detection and comparison of bacterial community composition in different trophic status lakes and *Sphagnum* peat bog



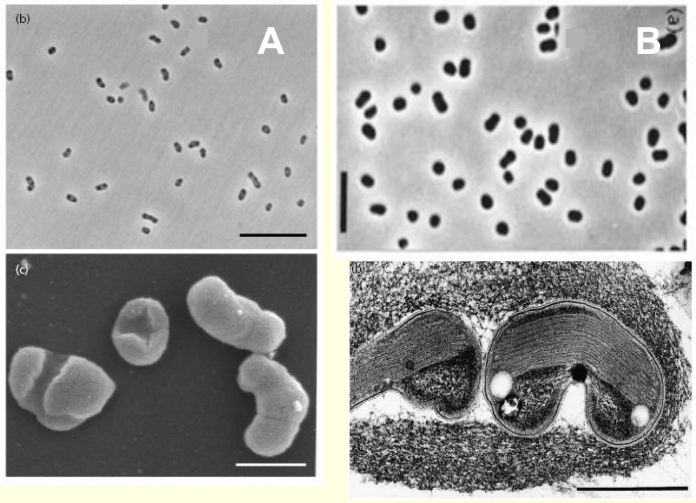




The structure of microbial communities in lakes and a bog differ significantly. Most interesting is high proportion of bacteria that cannot be identified by the currently known phylogenetic probes (designated on the diagram as "others") in acidic and oligotrophic aquatic ecosystems.

Novel methanotrophic bacteria from *Sphagnum* peat bogs

- **Methanotrophic bacteria** that inhabit acidic *Sphagnum* peat and reduce the flux of methane from northern wetlands is the most extensively studied group of microorganisms from *Sphagnum* wetlands. Currently, the list of taxonomically described methanotrophic bacteria that have been isolated from *Sphagnum* peat is restricted to members of the *Alphaproteobacteria* and includes representatives of the genera *Methylocella*, *Methylocapsa*, and *Methylocystis*. Interestingly, all novel species from wetlands capable of growth at low pH (4.2-5.5) and possess some unusual features or unique traits. For example, members of the genus *Methylocella* are facultative methanotrophs that are capable of growth on a number of multicarbon substrates including acetate, an important intermediate of carbon turnover in wetland ecosystems. By means of 16S rRNA-targeted fluorescently labeled oligonucleotide probes for specific detection of representatives of methanotrophic bacteria it has been shown, that they are numerically significant in acidic peat and can account for up to 11% of the total bacterial cell number.



Cell morphology of first acidophilic methanotrophic bacteria Methylocella palustris (A) and Methylocapsa acidiphila (B).

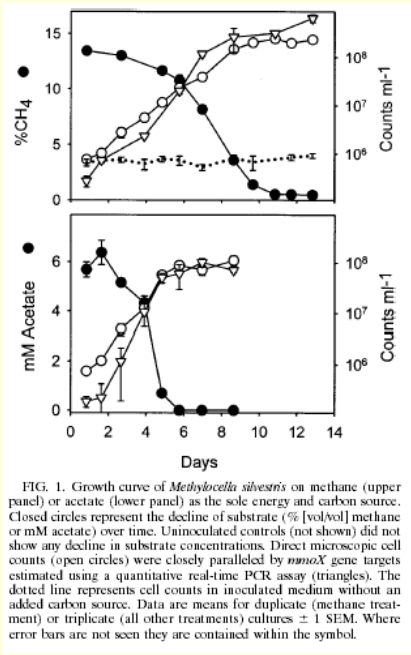


FIG. 1. Growth curve of *Methylocella silvestris* on methane (upper panel) or acetate (lower panel) as the sole energy and carbon source. Closed circles represent the decline of substrate (% [vol/vol] methane or mM acetate) over time. Uninoculated controls (not shown) did not show any decline in substrate concentrations. Direct microscopic cell counts (open circles) were closely paralleled by *mmoX* gene targets estimated using a quantitative real-time PCR assay (triangles). The dotted line represents cell counts in inoculated medium without an added carbon source. Data are means for duplicate (methane treatment) or triplicate (all other treatments) cultures ± 1 SEM. Where error bars are not seen they are contained within the symbol.

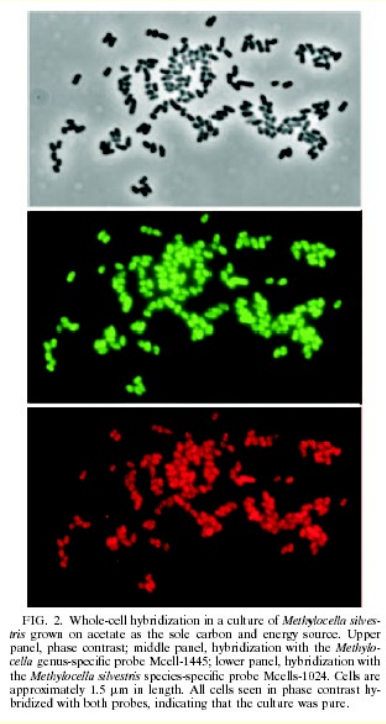
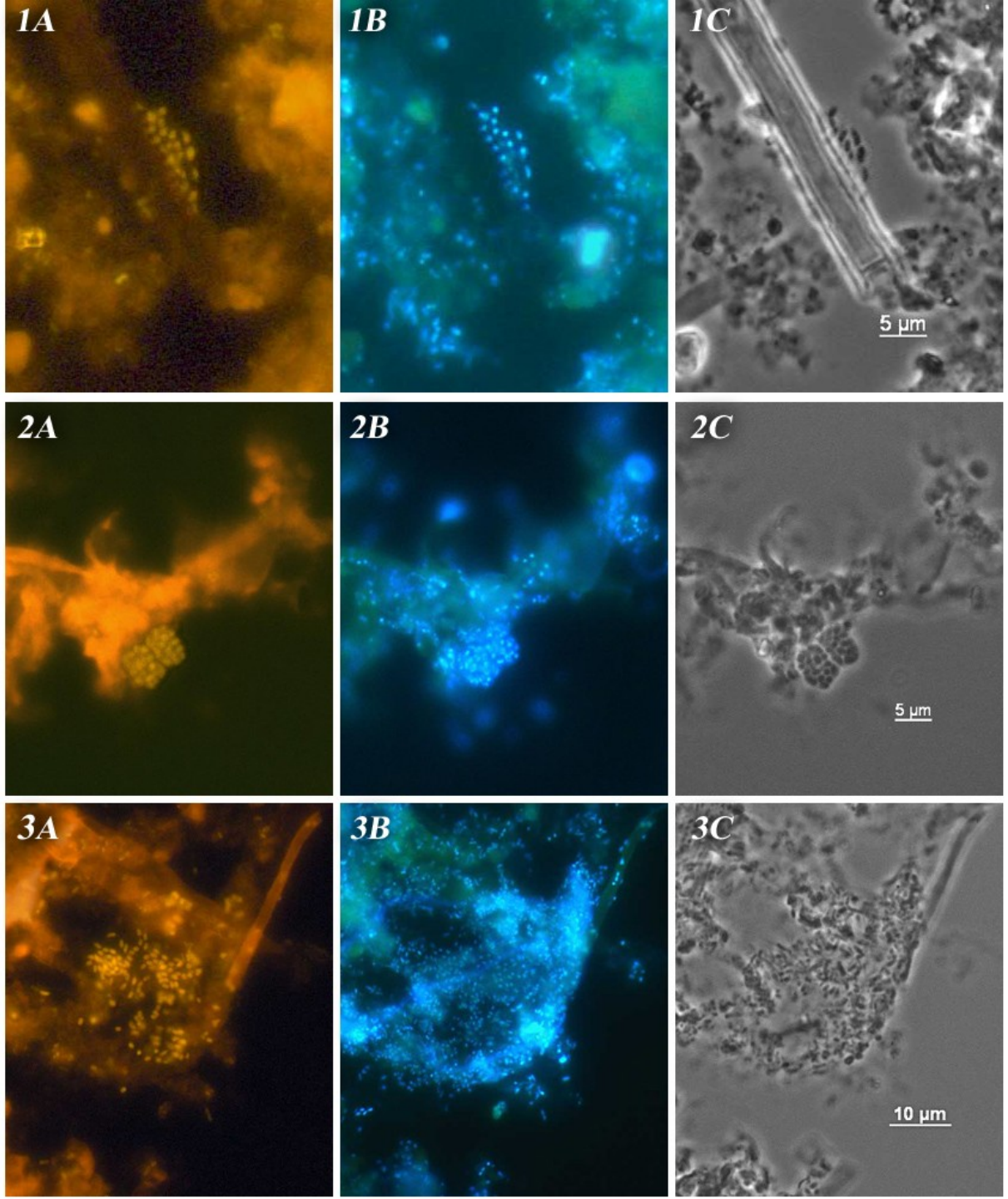


FIG. 2. Whole-cell hybridization in a culture of *Methylocella silvestris* grown on acetate as the sole carbon and energy source. Upper panel, phase contrast; middle panel, hybridization with the *Methylocella* genus-specific probe Mcell-1445; lower panel, hybridization with the *Methylocella silvestris* species-specific probe Mcells-1024. Cells are approximately 1.5 μm in length. All cells seen in phase contrast hybridized with both probes, indicating that the culture was pure.



Specific detection of representatives of the *Acidobacteria* in a peat sample by FISH:

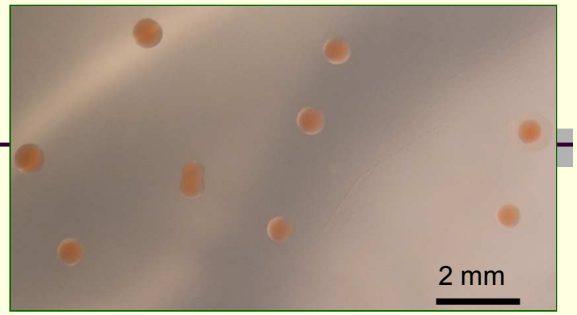
A - the epifluorescent micrographs of *in situ* hybridization with Cy3-labeled *Acidobacteria*-specific probe HoAc1402,

B - DAPI staining,

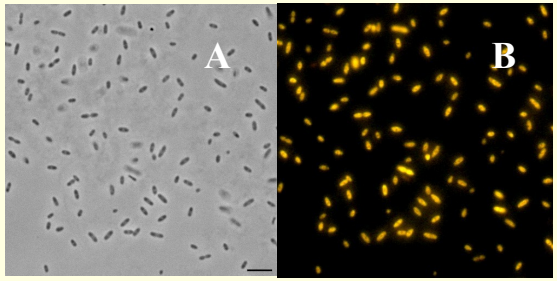
C – phase-contrast images

Representatives of the *Acidobacteria* from *Sphagnum* peat bogs

Acidobacteria form a coherent but highly diverse group within the bacterial domain



Colonies of acidobacteria on agar medium



Cells of strain T4: phase-contrast (A) and whole-cell hybridization with Cy3-labeled probe HoAc-1402 (B); bar, 5 μm.

16S rRNA gene-based dendrogram showing the phylogenetic relationship of peat clones to representatives of the *Acidobacteria*

Growth of acidobacteria at low pH and low temperatures

pH range:

Acidobacterium capsulatum (Kishimoto et al., 1991): **3.0 – 6.0**

Holophaga foetida (Liesack et al., 1994): **5.5 – 8.0**

Geothrix fermentans (Coates et al., 1999): no data

Strains T3 and T4 from *Sphagnum* peat: 3.0 - 6.5, μ (pH 3,5) = 0,05 h^{-1}

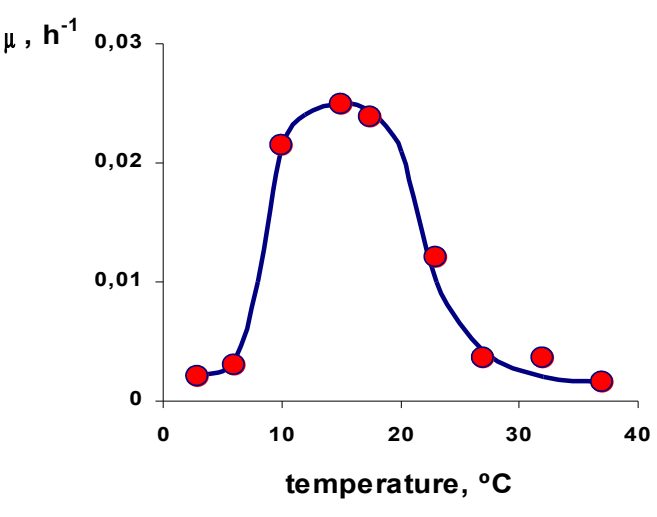
Temperature range:

Acidobacterium capsulatum : **+20 – +37°C**

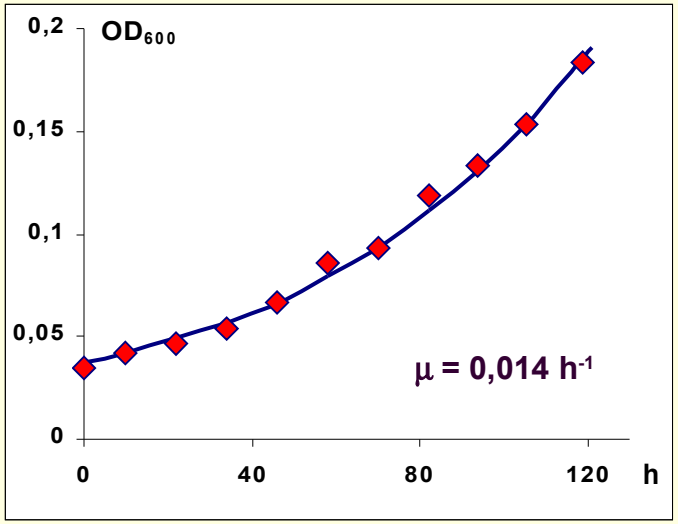
Holophaga foetida : **+10 – +35 °C**

Geothrix fermentans : **+35 °C** (opt)

Strains T3 and T4 from *Sphagnum* peat: +2 - +37 °C, optimum - +15 °C

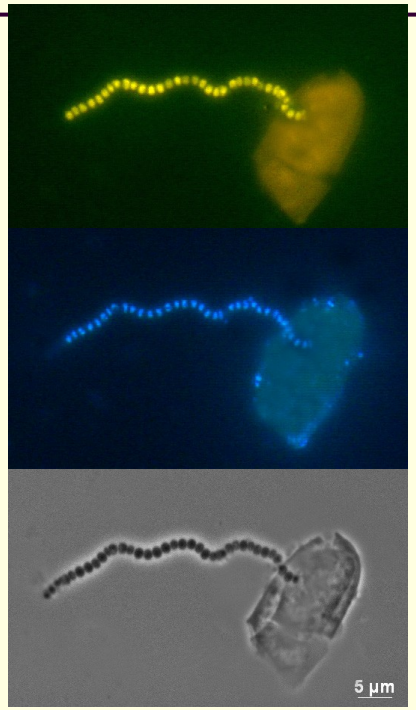


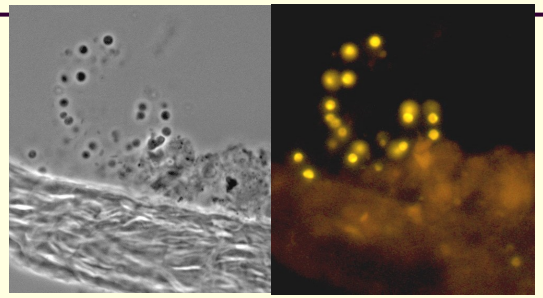
Temperature growth range of strain T4 (static conditions)

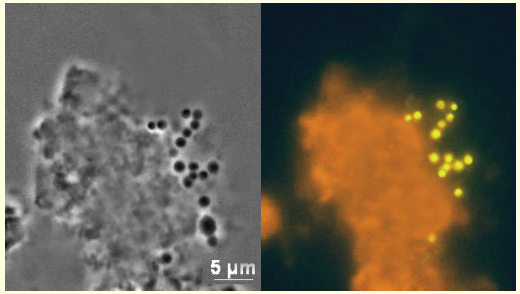


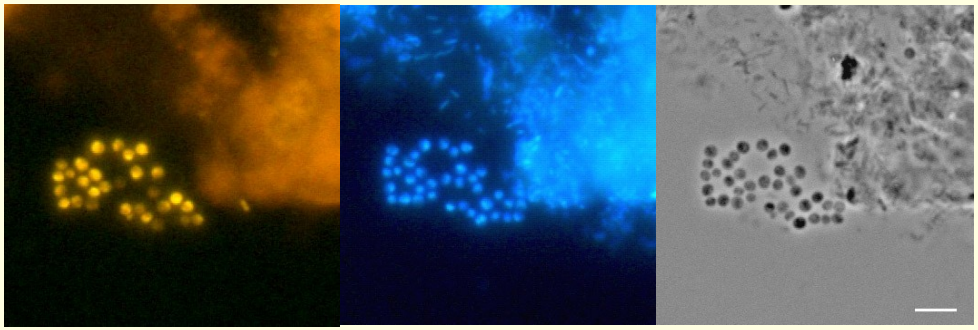
Growth of strain T4 at + 4 °C (incubation on a rotary shaker)

The *Planctomycetes* – one of the major bacterial groups in *Sphagnum* –dominated peatlands







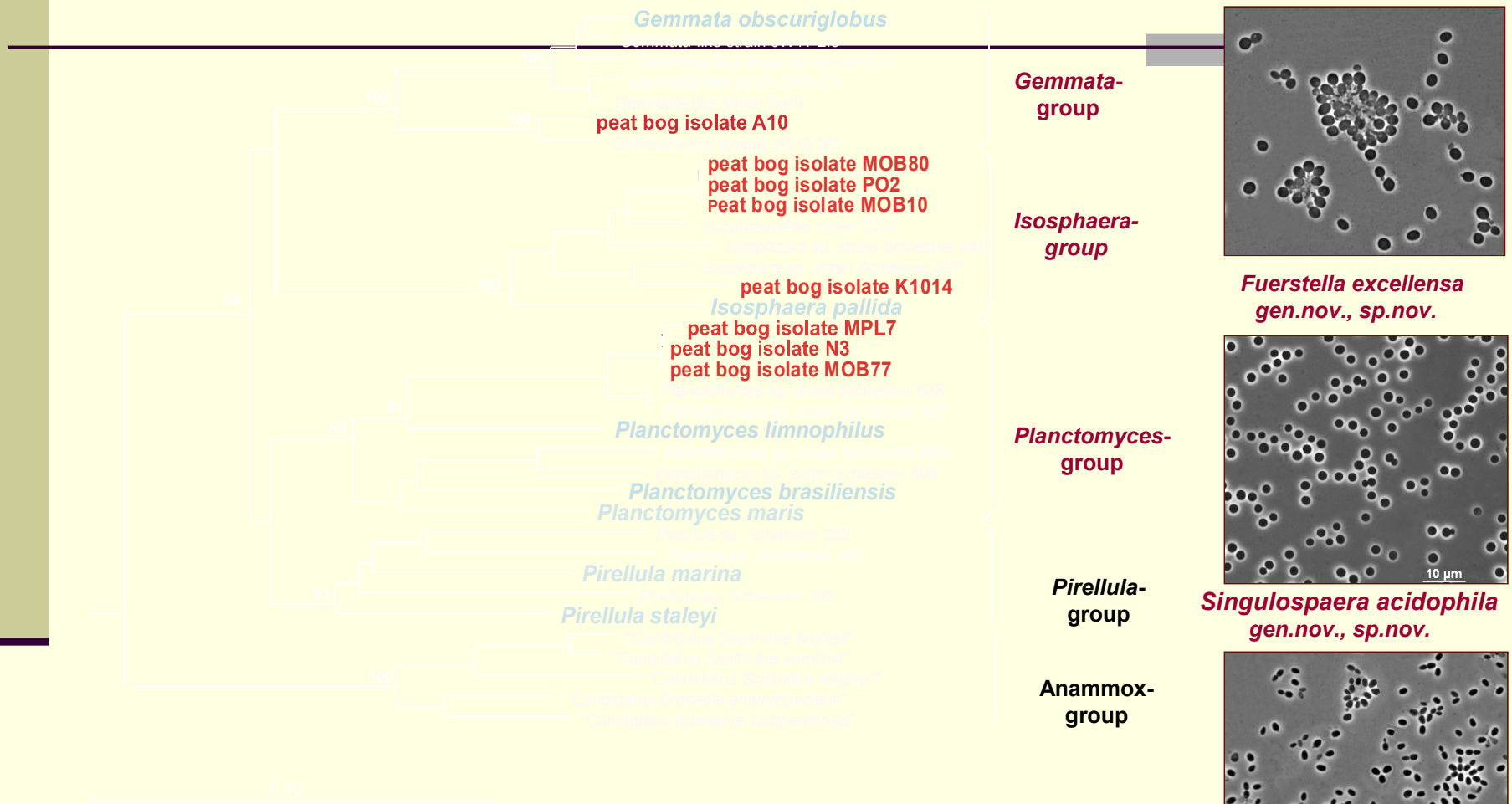


-The number of planctomycete cells in the upper (0-10 cm) oxic layer of the peat bog profile is in the range **0.4-2.0×10^{7}** cells g^{-1} of wet peat,

-Planctomycetes comprise **4 to 13%** of the total bacterial cell number determined in oxic layers of *Sphagnum* peat bogs by FISH

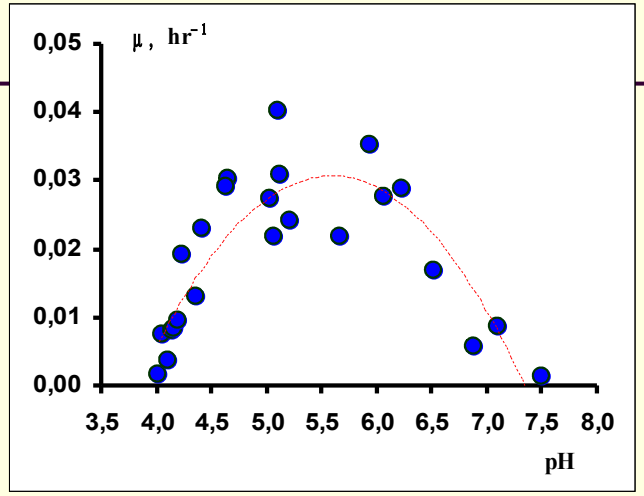
In situ detection of planctomycetes in *Sphagnum* peat by FISH with Cy3-labeled probes PLA46+PLA886

Novel acidophilic planctomycetes from *Sphagnum* peat bogs



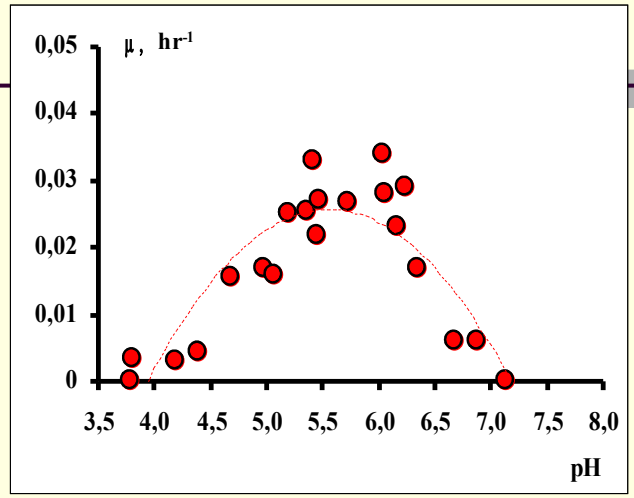
Isolates from *Sphagnum* peatlands possess only **87-89%** 16S rRNA gene similarity to taxonomically described planctomycetes

Growth of planctomycetes at low pH and low temperatures

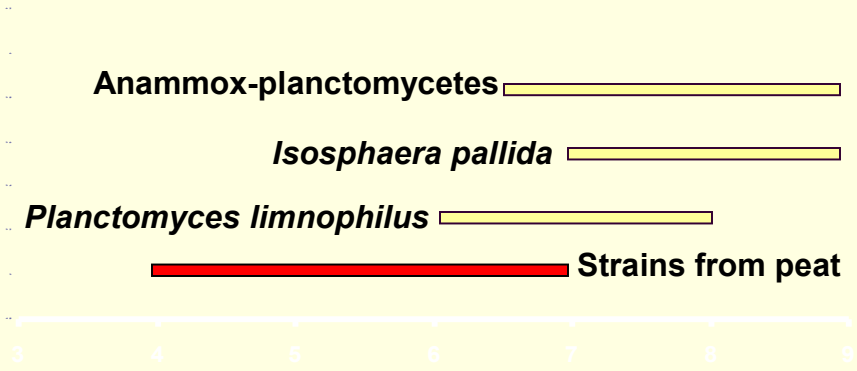


Planctomyces-like strain MPL7

pH range

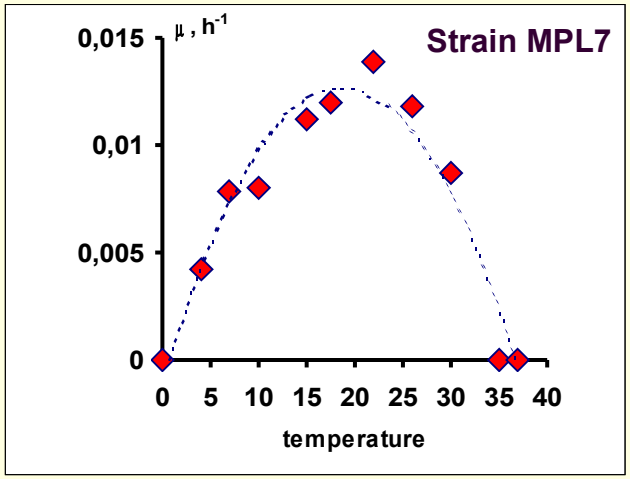


Isosphaera-like strain MOB10

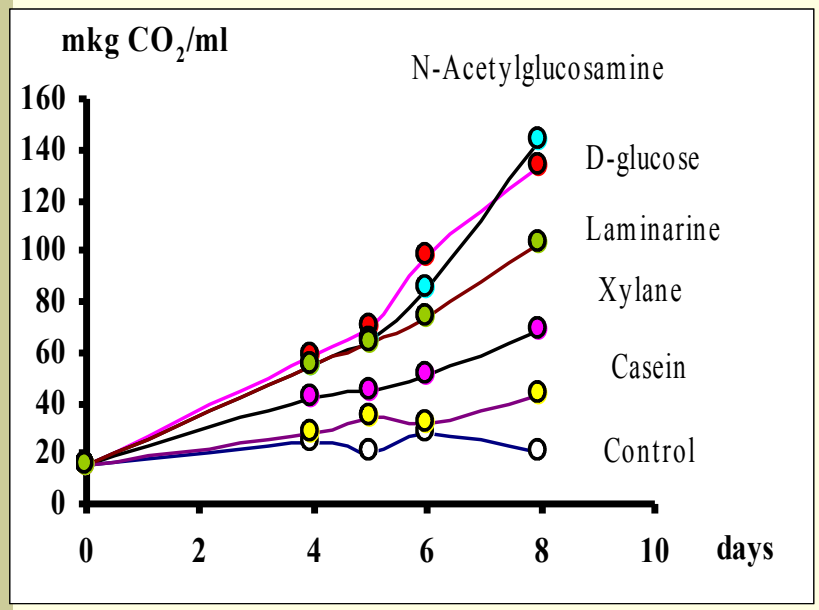


Comparison of pH growth ranges for planctomycetes from peat and earlier known planctomycetes

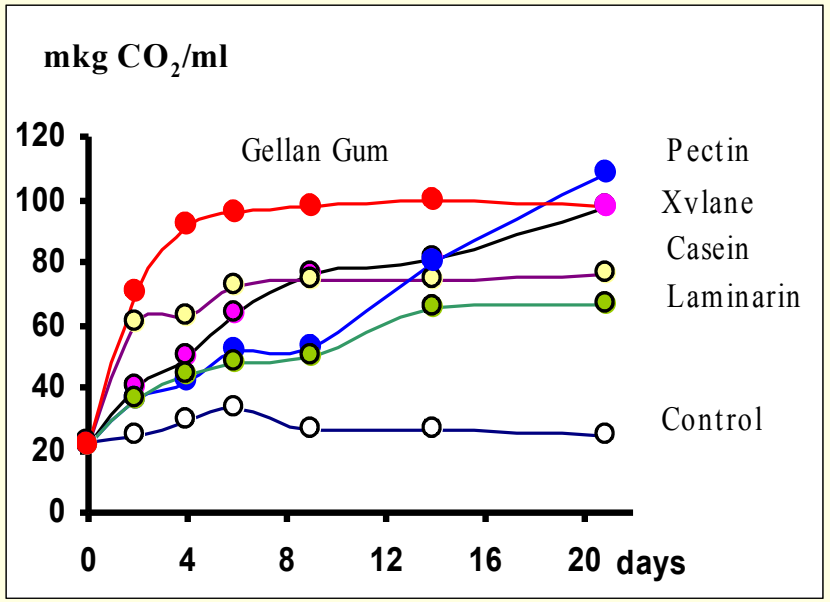
Temperature range



Are they capable of degrading biopolymers?



Isosphaera-like strain MOB10



Gemmata-like strain A10

The planctomycetes from *Sphagnum* peatlands are capable of degrading several biopolymers, such as pectin, laminarine, xylane suggesting their potential functional role in degradation of plant detritus *in situ.*

Conclusions:

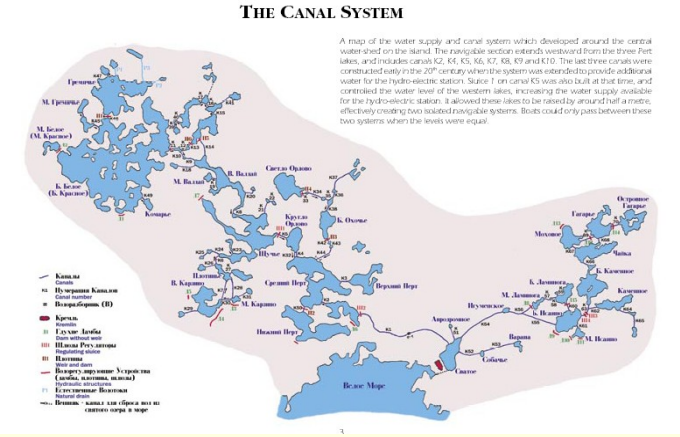
- ***Sphagnum*-dominated northern wetlands seem to represent a novel yet unexploited source for exploring the biodiversity of bacteria capable of growth at low pH, temperature and extremely low concentration of mineral nutrients.**

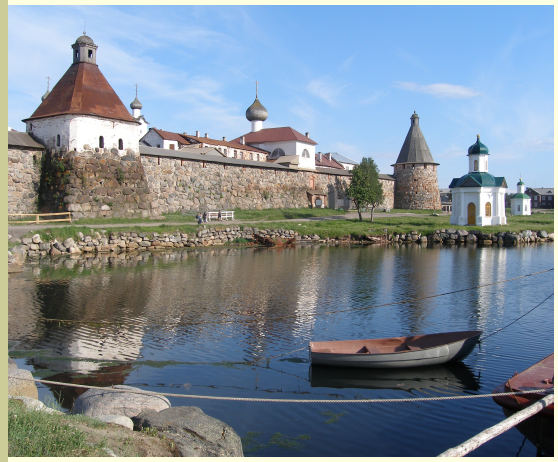
- **Isolation of acidophilic and psychrotolerant members of the *Acidobacteria* and the *Planctomycetes* from northern peatlands greatly expand our view of existing ecotypes of these poorly studied bacterial phyla.**

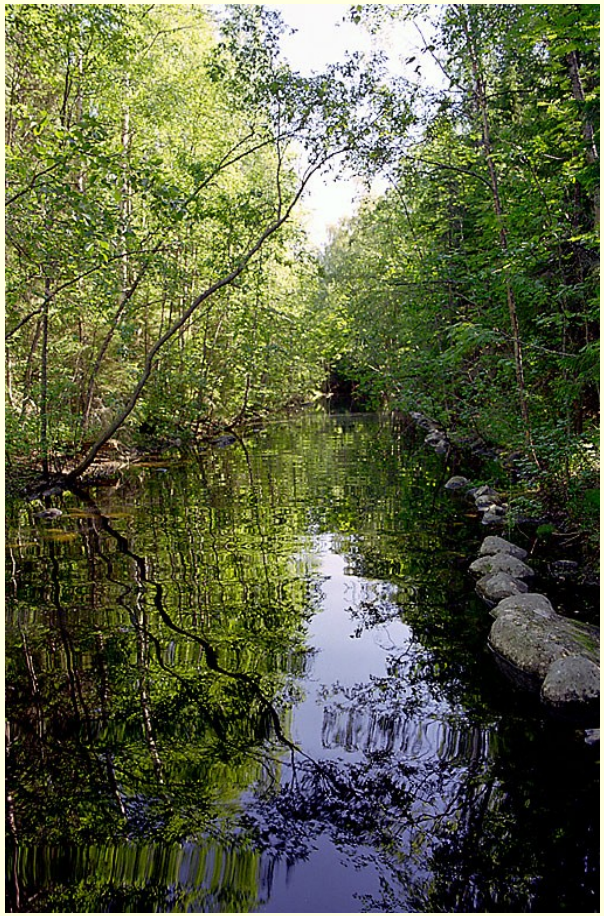
- **Many unique microorganisms that inhabit these cold and acidic ecosystems still elude our isolation attempts. This is a good challenge for future studies.**

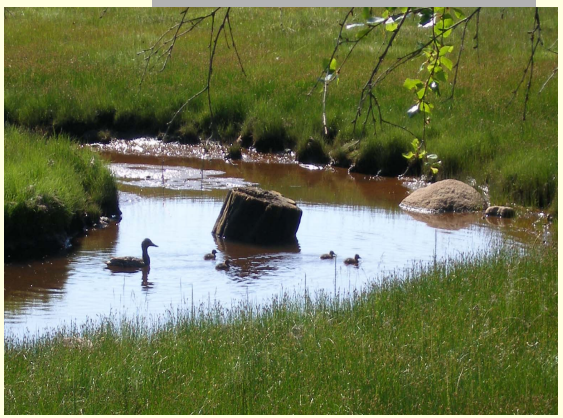
Solovki Canal System as example of wise use of wetland in landscape

Solovetsky Island does not have rivers, but it has hundreds of lakes. In the 16th century, monks started to connect lakes with canals. This system of lakes and canals stretching for 10 km was used as a fresh water resource, for transport and cargo transportation purposes. Nowadays, a good active and ecotour by rowing boats with opportunities to enjoy the nature f the Island is available.









Here are the members of a Russian „Wetland microbiology Team“:

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Anastasia Ivanova
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Alexey Vorob’ev
PhD student

Yulia
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diploma student

Andrey Kryazhev
diploma student

Thank you!!