USING OF THE SUCCESSION APPROACH FOR EVALUATION OF DIVERSITY OF BACTERIAL COMMUNITIES IN SOILS OF EASTERN ANTARCTICA (OASIS LARSEMANN HILLS)

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In recent years, intensive studies of the microbial diversity of Antarctic soils have been performed both with using classical approaches for cultivating bacteria on nutrient media and using modern molecular genetic methods [5, 6]. However, the analysis of thawed samples shows a relatively low number and diversity of bacteria on the traditionally used bacteriological nutrient media, with a relatively high number of bacterial cells, determined by luminescent microscopic method. In previously studied samples of Antarctic soils by fluorescent in situ hybridization (FISH), it was found that more than half of the number of identified bacterial cells within the domain *Bacteria* belonged to the phylum *Proteobacteria* [3], although during the plating of newly thawed samples of Antarctic soils, mainly Gram-positive bacteria grew.

It is known that one of the ways of survival of prokaryotes in inauspicious conditions is the conversion to viable but nonculturable state (VBNC) [1]. Probably, as a result of this, the analysis of newly thawed soil samples reveals a high content of filtering forms of prokaryotes (FFP) **(Fig. 1)**, which many researchers consider as special resting forms that ensure the viability of bacterial cells in the event of unfavorable environmental conditions [2, 4].

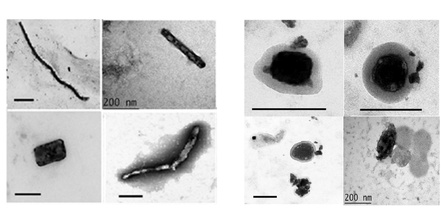

Figure 1 .Preparations of whole cells of FFP. The morphology of the FFP cells was studied in a transmission electron microscope (the scale corresponds to 200 nm)

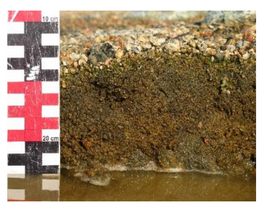

Figure 2. Soil profile with hypolithic horizon

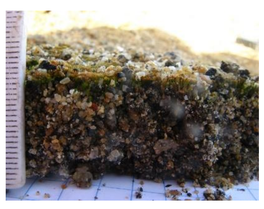

Figure 3. Soil profile developing under the moss cover

We applied a succession approach for evaluation the taxonomic diversity of cultivated heterotrophic bacteria in two soil samples from oases of East Antarctica, differing in content of organic matter.

The object of the study was soil samples selected and described by the participants of the 55th Russian Antarctic expeditions in the coastal part of East Antarctica in the oasis of the Larsemann Hills (Progress station).

The investigations were carried out in two samples of Antarctic soils differing in the content of organic matter. The first sample was taken from the mineral horizon B_1 (2-10 cm) of the soil profile where a hypolithic horizon was found **(Fig. 2)**, a low content of organic matter (0.14% carbon and 0.03% nitrogen) and a field moisture content of 6% was noted in the sample. The second sample is selected from the moss cover of *Ceratodon purpureus* and represents the B_{algae} horizon (1 - 2 cm) with inclusions of unicellular algae in the form of biofilms **(Fig. 3)**, this horizon was characterized by a higher content of organic matter (0.41% carbon and 0.05% nitrogen) and a moisture content of 7.2%.

Succession was initiated by moistening and subsequent incubation in two temperatures: + 5° and + 20°C, i.e. in conditions simulating a warm season in Antarctic region. Total number of prokaryotes and FFP as well as the number and taxonomic diversity of the heterotrophic bacterial complex were determined in the course of the model experiment. In all cases, the maximum of the total number of bacteria was fixed on the 14th day, the minimum at the beginning (the 1st day) and at the end (the 160th day) of the experiment. Contrariwise the number of FFP was maximal at the beginning of the experiment and minimal - on the 14th day **(Fig. 4, 5)**. The dynamics of the number of bacteria and FFP makes it possible to assume that "revitalization" of Antarctic soils by moistening and incubation at positive temperatures promotes the transition of resting cells forms to an active, viable state.

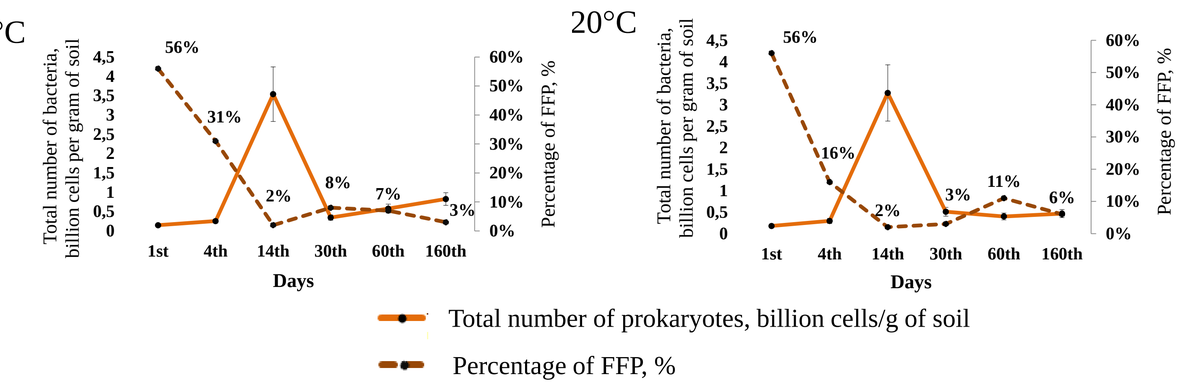

Figure 4. Dynamics of the total number of bacteria and the proportion of FFP in the course of succession in mineral horizon B_1

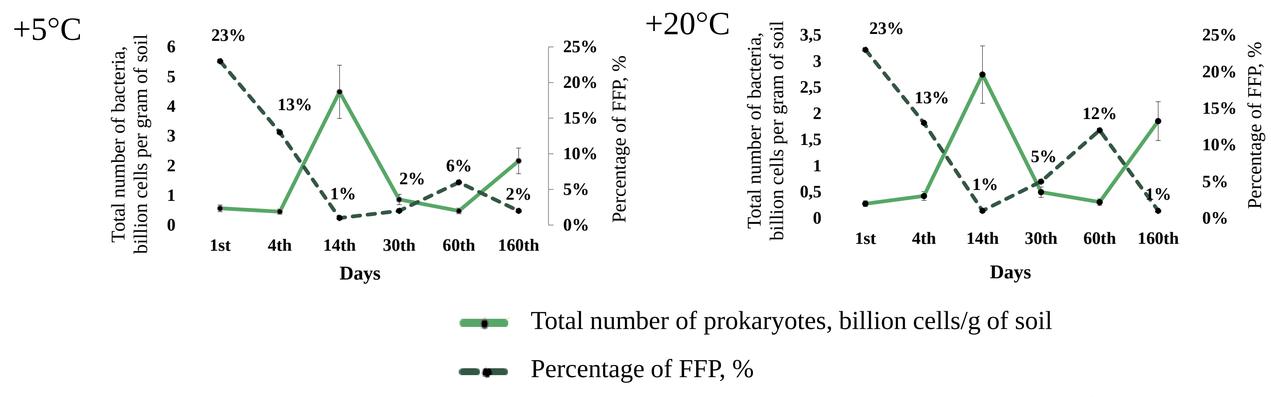

Figure 5. Dynamics of the total number of bacteria and the proportion of FFP in the course of succession in the B_{algae} horizon

Table 1. Structure of saprotrophic bacterial complex in mineral horizon B_1

Succession stage	Incubation temperature	Dominants	Group of medium abundance	Minor components
0 day	5°C	*Arthrobacter, Caulobacter*	*Bacillus*	*Micrococcus*
	20°C	*Caulobacter, Arthrobacter*	*Beijerinckia, Rhodococcus*	*Cellulomonas, Cytophaga, Micrococcus*
14th day	5°C	*Arthrobacter*	*Cytophaga, Proteobacteria, Micrococcus*	*Bacillus, Caulobacter, Streptomyces*
	20°C	*Arthrobacter, Proteobacteria*	*Proteobacteria, Micrococcus, Myxococcus, Polyangium*	*Proteobacteria, Rhodococcus, Myxococcus, Strepromyces*
60th day	5°C	*Arthrobacter*	*Cytophaga, Proteobacteria, Micrococcus*	*Bacillus, Sphingomonas*
	20°C	*Arthrobacter, Bacillus*	*Proteobacteria, Micrococcus* sp., *Myxococcus* sp., *Polyangium* sp.	*Cellulomonas, Cytophaga, Ralstonia, Rhodococcus*

In the course of succession, the number and taxonomic diversity of the heterotrophic complex of bacteria cultivated on the glucose-peptone-yeast nutrient medium also changed. The genus *Arthrobacter* dominates at all stages of the model experiment. The diversity of grown bacteria increased in groups of average abundance and minor components from the 14th day of experiment and further along the succession **(Table 1, 2)**. During the experiment, Gram-negative bacterial strains were also isolated and identified by 16S rRNA gene sequence analysis. All of them were affiliated to the phylum *Proteobacteria*, genera *Sphingopyxis*, *Bosea*, *Sphingomonas*, *Brevundimonas*, *Ralstonia*, *Delftia*, *Variovorax*, *Stenotrophomonas*, *Pseudomonas*, *Acinetobacter* **(Table 3)**. A high similarity to a particular species was not found for many studied strains which makes it possible to assume that they belong to new species of bacteria.

Table 2. Structure of saprotrophic bacterial complex in the B_{algae} horizon

Succession stage	Incubation temperature	Dominants	Group of medium abundance	Minor components
0 day	5°C	*Cytophaga, Proteobacteria, Bosea, Myxococcus*	–	–
	20°C	*Cytophaga, Bacillus, Myxococcus*	–	–
14th day	5°C	*Arthrobacter, Ralstonia*	*Streptomyces, Polyangium*	*Caulobacter, Myxococcus, Spirillum, Proteobacteria*
	20°C	*Arthrobacter, Cytophaga*	*Variovorax, Brevibacterium, Proteobacteria, Mucilagini-bacter, Myxococcus*	*Delftia*
60th day	5°C	*Arthrobacter, Polyangium*	*Cytophaga, Proteobacteria*	*Beijerinckia*
	20°C	*Arthrobacter, Proteobacteria, Myxococcus*	*Arthrobacter, Cellulomonas, Cytophaga, Ralstonia*	*Polyangium*

Table 3. Taxonomic specification of gram-negative bacteria identified by analysis of 16S rRNA gene sequences

Alphaproteobacteria	*Betaproteobacteria*	*Gammaproteobacteria*
Sphingopyxis bauzanensis	*Ralstonia* sp. (three strains)	*Stenotrophomonas* sp.
Bosea thiooxidans (tree strains)	*Ralstonia picketti* (two strains)	*Pseudomonas* sp. (two strains)
Sphingomonas sp. (eight strains)	*Delftia acidovorans*	*Pseudomonas vancouverensis*
Bosea sp.	*Variovorax* sp. (two strains)	*Acinetobacter* sp.
Brevundimonas sp.		

Thus, we suppose that the application of the succession approach allows to more fully characterize the taxonomic diversity of the heterotrophic bacterial complex and to identify a wider range of the genera of Gram-negative bacteria.

The use of a succession method can be recommended both for a comprehensive study of bacterial complex of extreme biotopes and for other studies in which it is necessary to obtain the maximum diversity of cultured bacteria from natural samples.

References

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