ISSN 0236-2457

ASTRONOMICHESKII TSIRKULYAR

Published by the Eurasian Astronomical Society
and Sternberg Astronomical Institute

No. 1618, 2014 November 30

Study Of Functioning of Bacterial Complexes in East Antarctic Soils As a Model for Astrobiology[1]

A.V. Yakushev[*], N.A. Churilin[*], V.S. Soina[*], A.G. Kudinova[*]
E.A. Vorobyova[*, **], N.S. Mergelov[***]

[*] *Faculty of Soil Science, Moscow State University*
Leninskie Gory, Moscow, 119234 Russia

E-mail: a_ yakushev84@mail.ru

[**] *Space Research Institute, Russian Academy of Sciences*
Profsoyuznaya ul. 84/32, Moscow, 117997 Russia

[***] *Institute of Geography, Russian Academy of Sciences*
Staromonetnyi per. 29, Moscow, 109017 Russia

Received September 16, 2014

Abstract. Studies of bacterial communities in the samples of Antarctic soils by different methods showed that, both in liquid soil suspensions and *in situ*, microbial complexes are functioning presumably by forming biofilms – the phenomenon that is more expressed in such habitat than in soils of temperate zones. Functional (trophic) diversity and physiological state of hydrolytic bacteria was studied in the samples at the upper layer (0–2 cm) of gravel pavement with algae, in the underlying peat horizon (2–4 cm) with inclusions of dead biomass and its underlying mineral horizon (4–10 cm) with signs of fungal mycelium. The investigated samples of Antarctic soils revealed different trophic diversity and the maximum specific growth rate on mineral medium with different biopolymers as the sole carbon source (starch, chitin, pectin, xylan, dextran-500, tween-20, casein); this can testify to differences in the physiological state of hydrolytic bacteria in various soil horizons and their readiness for growth. The most remarkable characteristics of the studied Antarctic soil as compared to the soils of temperate zone, was the unusual ability of hydrolytic community to consume chitin in the mineral horizon; this can be explained by the presence of fungal mycelium. Also, an almost complete lack in consumption of tween-20 (a water-soluble analogue of fat) by bacterial community of Arctic soil horizons are not explained and needs further verification. The higher functional diversity was detected in the upper horizon of the gravel pavement, which "protects" microorganisms from exposure to extreme temperatures, UV radiation, and desiccation, but the maximum specific growth rate was higher in the lower mineral horizon; this can be explained by the specificity of bacterial colonizing processes and unique formation of Antarctic soil microprofiles in the Larsemann oasis. The obtained data indicate a specific environmental strategy in the samples of Antarctic soils: development in lower mineral horizons of microorganisms with a high metabolic readiness to life revival and high maximum growth rate.

[1] Talk presented at the Interdisciplinatory Colloquium on Cosmic Factors of Evolution of the Biosphere and Geosphere, Moscow, May 21–23, 2014.

Introduction

Low temperature is a predominant environmental characteristic of interstellar space, asteroids, meteors, and our Solar System, including most of the planets and their satellites. An understanding of the impacts that low temperature affects on preservation and evolution of biological organisms is, therefore, integral to our knowledge in astrobiology. Microbial adaptation to low temperature in Antarctic soils and permafrost is widely studied now as the most representative models for the search of life preservation in extraterrestrial cryogenic habitat.

Antarctic soil investigations concentrate mainly on soil genesis, development of relatively detailed soil maps and molecular-genetic analysis of microbial components in such soils (Vishniac H.S., 1993; Yergeau E., et al., 2007). Molecular-genetic methods showed the diversity of bacteria in Antarctic soils, but these results do not reveal biological activity, physiological status, and stability of microbes in external environment. For understanding the functioning of bacterial ecosystems, it is necessary to find out whether the bacteria are able to perform in extreme conditions one of the important functions in biosphere, the decomposition of biopolymers.

The subject of the research was to study the total count of bacterial cells *in situ*, physiological diversity, and physiological state of hydrolytic bacteria, their environmental strategy in the samples of Antarctic soils.

Materials and methods

The samples for the study were taken from the interhill wet valleys area of Larsemann Hills (East Antarctic Coast). The bottoms of inter-hill valleys show maximum biota concentration and highest bio- and soil diversity. Moss, lichen, and algae ground covers are formed here as well as algal-bacterial mats, and microorganisms develop various soil profiles in sandy granitoid sediments.

The total count of bacterial cells was obtained by fluorescence microscopy (Zeiss Axioskop 2) and staining with acridine orange (AO). Morphological analysis of microbial populations was carried out using a scanning electron microscope (JSM-6610LV with X-ray microanalyzer Oxford Instruments).

Functional (trophic) diversity and physiological state of hydrolytic bacteria were studied in the samples at the upper layer (0–2 cm) of gravel pavement with algae, in the underlying peat horizon (2–4 cm) with inclusions of dead peat biomass and its underlying mineral horizon (4–10 cm) with signs of fungal mycelium (Figure 1).

Determination of the functional (trophic) diversity of bacteria in soil samples and their maximum specific growth rate on nutrient media containing various organic substances were carried by a specific method (Jakushev et al., 2011, 2012) based on the determination of the duration of the lag phase of growth of microorganisms isolated from natural substrates in the culture media, which may depend not only on the culture conditions, but also on the physiological state of microorganisms in natural substrates before their selection. It is assumed that the shorter the lag phase of growth of the microorganism in a culture medium, the more active the body was in the natural environment. The physiological state of the microorganisms was determined from the initial growth curve (lag phase and the phase of unlimited growth) on nutrient media. The method is based on synthetic chemostate model

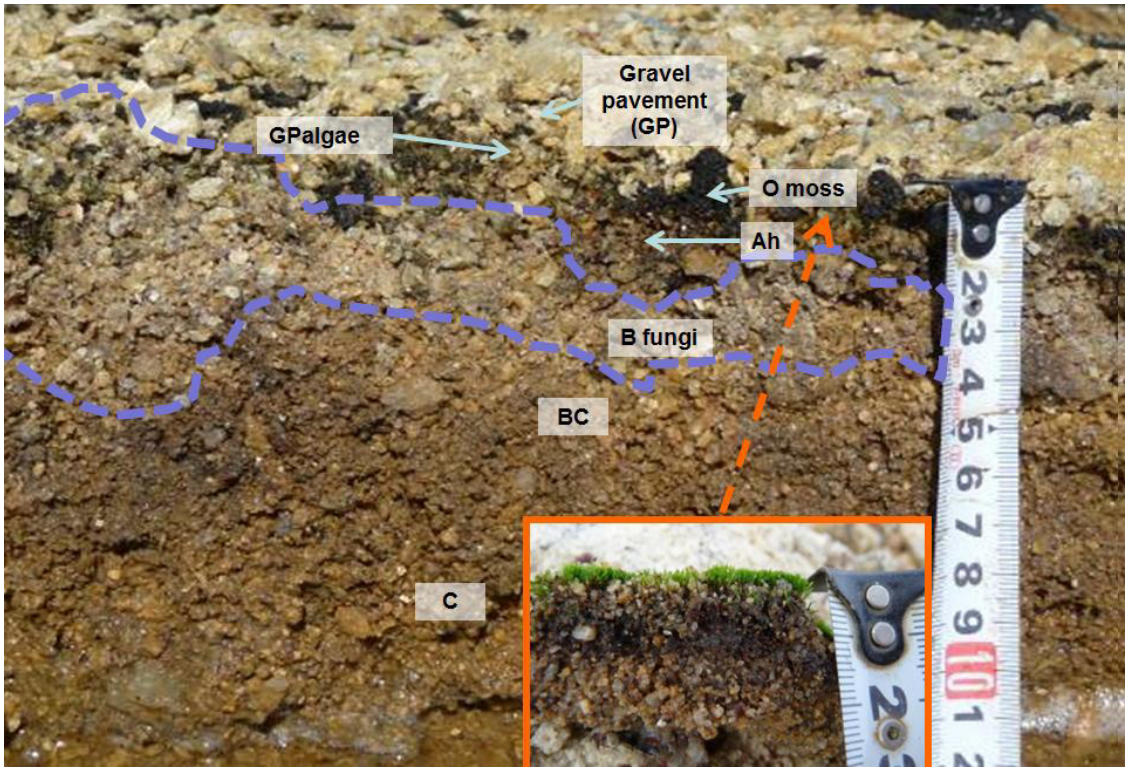

Figure 1: Profile of the investigated soil horizons. Soil at the wet valley of Larsemann Hills oasis beyond the meltwater flow. Source of moisture: snow patches. (Site M3): GP – gravel pavement, GPalgae – green algae and cyanobacteria in the sandy bedding of gravel pavements.

for a simplified description of the lag phase and phase of unlimited growth of a batch culture of microorganisms.

Growth equation is as follows:

$$x(t) = x_0 \left(1 - \rho_0 + \rho_0 e^{\mu_m t}\right)$$

where $x(t)$ is the concentration of microorganisms in the culture at time t, x_0 is the initial concentration of microorganisms, μ_m is the maximum specific growth rate (h^{-1}), ρ_0 is the initial value (value in soil) of the physiological state of the growing culture.

The method can also determine the functional (trophic) diversity of microbial communities (number of media on which growth was recorded) and growth kinetic parameter – the maximum specific growth rate (μ_m), which reflects the environmental strategy of bacterial growth.

All procedures were performed under the sterile conditions. Each time the analysis was provided for the control of sterility. The scheme of the experiment is shown in Fig. 2.

Homogenization and desorption of microorganisms were carried out in an aqueous slurry (1:1) for 20 min at 2000 rev/min on a shaker "vortexes", model "Multi Reax", firm "Heidolph". Fungal growth in suspension was inhibited by adding an antibiotic nystatin in a concentration of 0.05%. The excess of substrate particles were removed by centrifugation at 3200g for 5 minutes. The concentration and composition of the cultured microorganisms in supernatant at the step of initial seeding on the substrate was determined on glucose-peptone-yeast agar

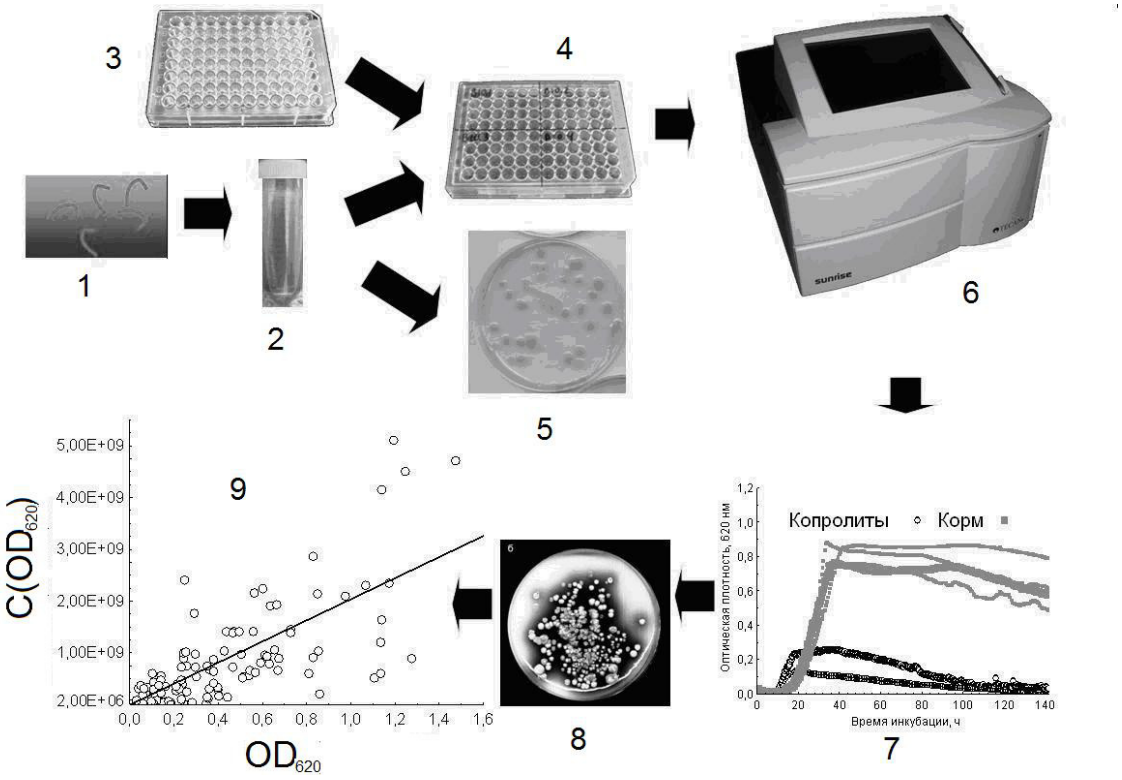

Figure 2: The framework for the method of complex structural and functional characteristics of microbial populations.

medium in a conventional manner. The supernatant was added to 100 μl in 96-well cell culture flat-bottomed plates with a lid, which has made a set of different liquid media with 100 microliters.

The following substrates (polymers) for different research goals were selected as the sole carbon source:

– starch (glucose reserve polysaccharide of plants and green algae), carboxymethylcellulose,

– chitin (aminopolysaccharide part of the cell walls of fungi and the shells of arthropods),

– pectin (polygalacturonic acid methyl ester) performing the function of the adhesive in the cell walls of plants),

– xylan (most abundant polysaccharide from the group of hemicelluloses, a part of the filler plant cell wall polymer xylose), dextran-500 (branched bacterial polysaccharide aD-glucopyranose, is a part of the mucous capsule of microorganisms),

– Tween-20 (water-soluble analogue of fats),

– casein (milk protein).

Two types of control were used: for the uptake of biopolymers the mineral salt solution without introducing organic matter was used, and for the sterility a version without inoculation of cells containing biopolymers by an aqueous suspension of soil samples was taken.

The bacterial growth on a medium with different biopolymers was measured by an optical method using enzyme immunoassay analyzer Sunrise company Tecan (Switzerland). As on the liquid nutrient media mixed populations usually grow, the obtained parameters considered to be averaged.

The identification of taxonomic groups in associations grown at various biopolymers was determined after 215 hours of cultivation at +250°C by plating on yeast-glucose-peptone agar medium using culture-morphological characteristics.

The description of the lag phase (the stage of preparation of microorganisms to growth) and of the phase of exponential growth (the growth phase when food is in abundance) in the complex model of growth of a batch culture allows us to characterize the kinetic parameters of bacterial growth. The conducted experiments use a parameter of metabolic readiness to consume a particular polymer occurring microbial association $\gamma = -\ln(\rho_0) = \mu_m t_{lag}$. This value is directly proportional to the associated t_{lag}—duration of the lag phase.

Results and Discussion

The total number of bacterial cells counted by staining with acridine orange (AO) was rather high for the Antarctic habitat and varied in the range of more than 10 mln cells/g. in the samples from different sites (Fig. 3).

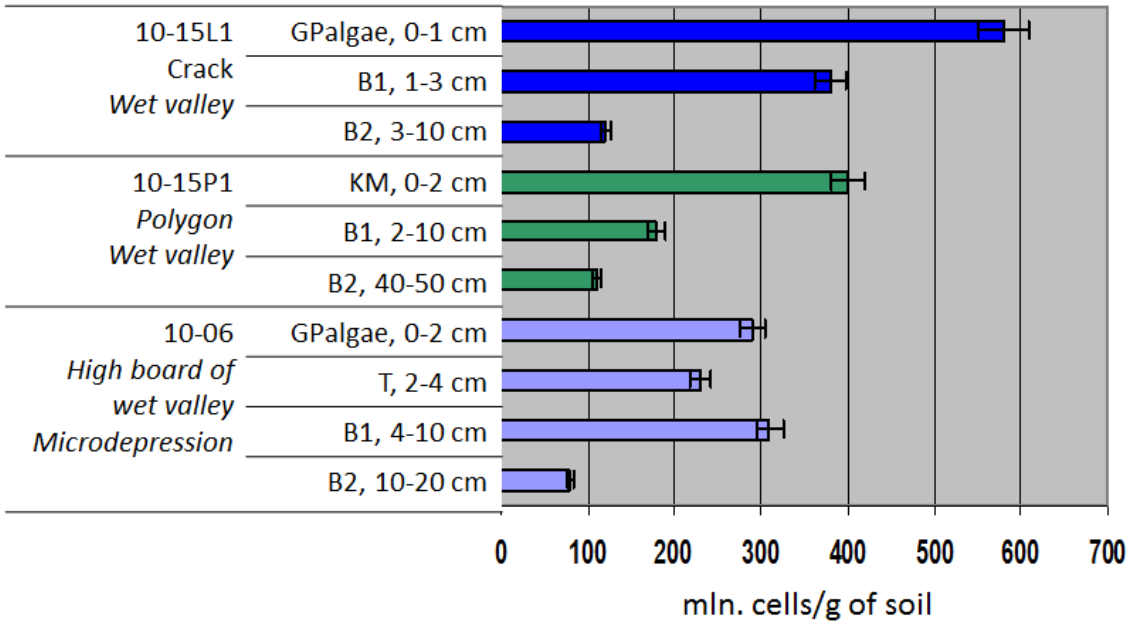

Figure 3: Total number of bacteria, AO staining, sites 10-15L1, 10-15P1, 10-06.

The increased number and proportion of viable cells (counted in number of colony forming units — CFU on nutrient medium per gram of soil sample) was observed in the fine earth directly under the gravel pavement. Such habitats (sand beddings with moss pads, algae, and micromycetes colonies) are most favorable for the development of bacteria, as sheltered by the gravel pavement from wind corrosion, dehydration, and aggressive UV radiation, but at the same time they are close to the surface, well warmed by pavement insolation, and are fed by the melting snowfields.

Study of viable bacteria in Antarctic soil horizons, where we investigated functional (trophic) diversity and physiological state of hydrolytic bacteria, revealed a sharp decline in the number of colony forming units (CFU/g) with the depth – from the upper to the lower mineral horizon (Fig. 5).

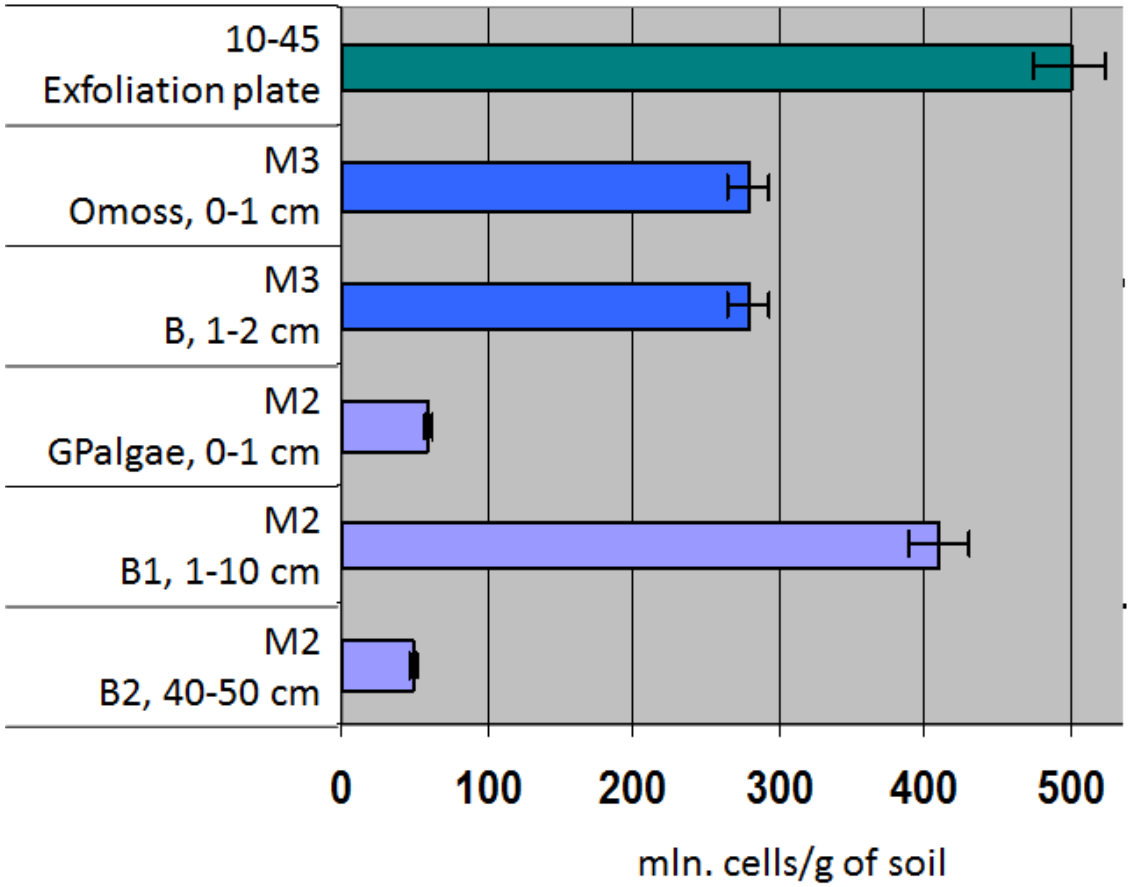

Figure 4: Total number of bacteria, AO staining, sites 10-45, M3, M2.

The most remarkable phenomenon in the studies of bacterial complexes of the investigated Antarctic soil horizons is that, unlike soils in temperate zones, which are characterized by the presence of free-swimming planktonic forms, in liquid associations of Antarctic soils dominated cells that formed biofilms (particularly, Bacillus cereus var. mycoides). The study of microbial complexes *in situ* by SEM also revealed biofilms that play an important role for preservation of life in such an extreme habitat (Fig. 6).

Physiological diversity of bacteria – hydrolytic, assessed by the number of culture media with polymers in which there was an increase after the introduction of the soil suspension. In this modification of the threshold sensitivity of >10 cells are able to grow on the polymer per gram of soil.

The study of the functional diversity of microbial communities revealed differences in the utilization of substrates by bacterial communities from different horizons. For instance, it was shown a reduction in the functional diversity of communities of hydrolytic bacteria.

In a sample of the upper horizon of gravel pavement all examined substrates were taken up by microorganisms (starch, pectin, dextran-500, casein, carboxymethylcellulose (CMC), chitin, xylan), while in peat and mineral horizons the amount of consumed substrates decreased to 5 and 3, respectively, in the peat (starch, pectin, dextran-500, casein, chitin) and mineral horizon (chitin, starch, casein).

Figures 7 illustrate the growth in a mineral medium with different biopolymers. It was

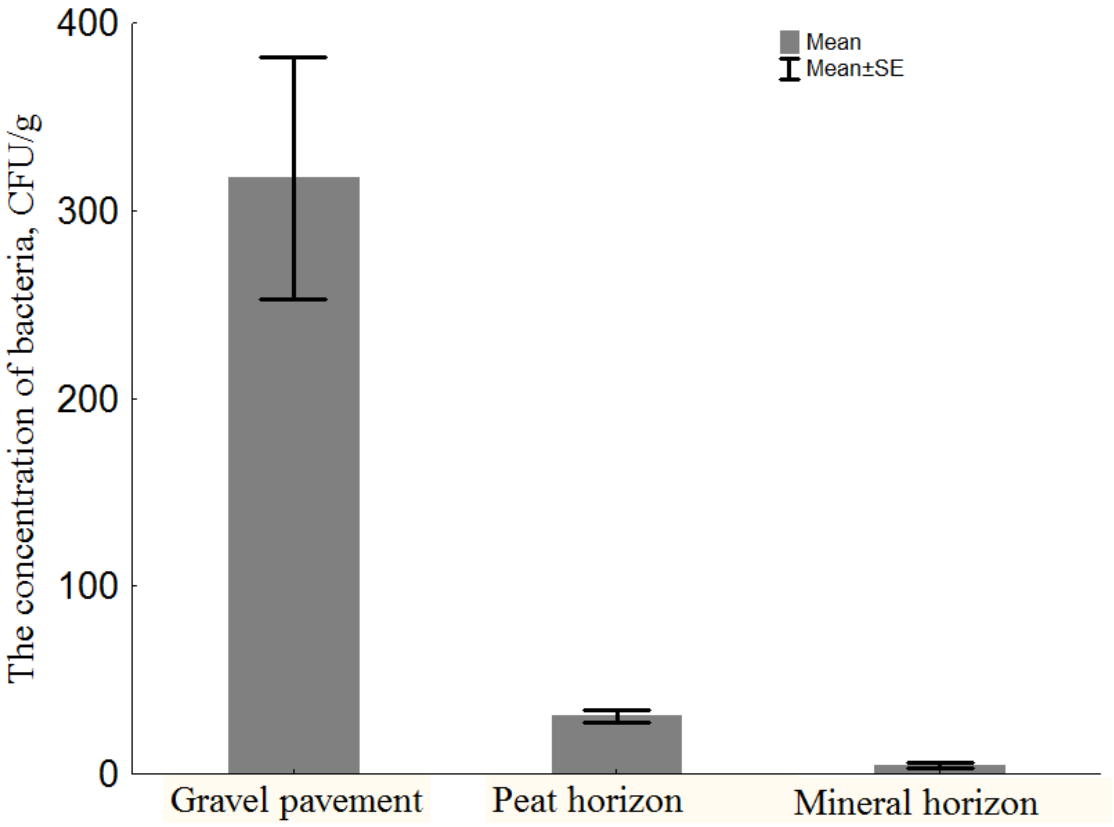

Figure 5: Number of colonyforming units (CFU/g) in the samples of various horizons.

shown that the utilization of biopolymers such as xylan, dextran is revealed only in the upper horizon (gravel pavement), where cellulolytic bacteria apparently dominated; this can be explained by the presence of algae in this horizon.

Chitin was consumed in the subsurface peat horizon due to high abundance of fungal mycelium containing chitin and visible by the naked eye. For hydrolysis of the chitin in the liquid medium are responsible bacteria of the genus Bacillus. The consumption of substrates such as Tween-20 and pectin was noted in two horizons. Thus, pectin was consumed by bacteria only in peat horizon and a sample of gravel pavement, and a twin – 20 only in peat horizon.

Paradoxical poor growth of bacteria in the medium with tween-20 (analogue of water-soluble fats), which are usually regarded as easy disposition substrate, may be considered as specific feature of Antarctic bacteria. Finally, bacterial growth on such substrates as casein, starch and chitin was detected in all horizons.

The study of the physiological state of bacteria in the samples of soil in terms of their growth activity on examined substrates at the initial period of growth and at the stage of maximum specific growth rate revealed the following.

Figures 8 demonstrate the diagrams of metabolic activity and maximum specific growth rate of bacteria for each substrate, and Figures 9 show the total value of metabolic readiness for growth on all the substrates. High readiness to grow on different substrates was revealed in the sample on the gravel pavement, especially on casein, dextran, and pectin, but not

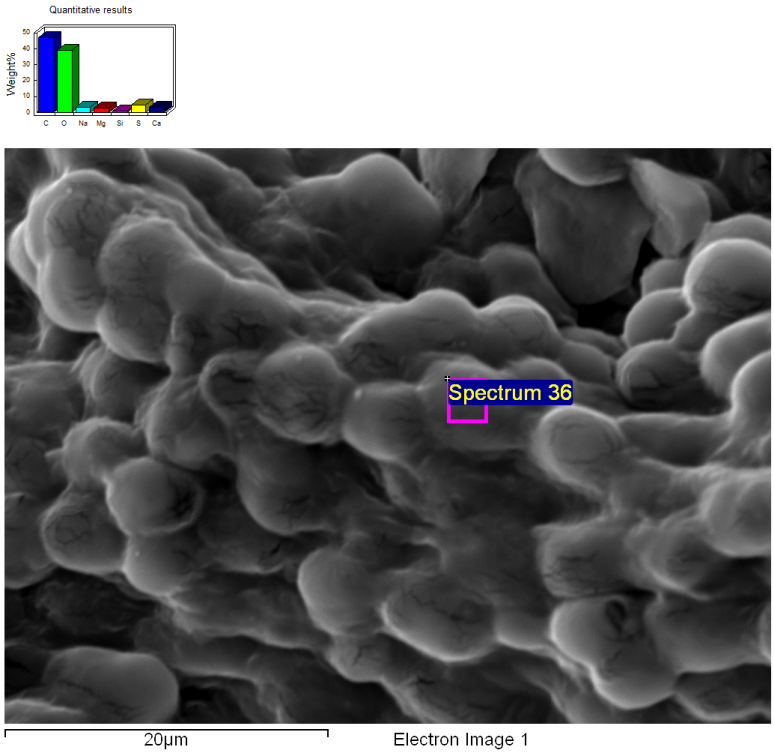

Figure 6: Biofilms in the sample of Antarctic soils (the fine earth directly under the gravel pavement).

starch. The maximum specific growth rate is higher in the mineral horizon. This manifests itself on substrates casein and starch, and it is connected with the fast growing bacteria.

The physiological state of the bacteria in the soil, which was estimated by the metabolic preparedness growth of microbial associations (γ), suggests naturally greater microbial activity in the upper horizon stone bridge (see Fig. 8). An interesting increase in the maximum specific growth rate of bacterial associations in the liquid media after inoculation suspension of the lower horizon (Figure 9), which indicates an increase in the proportion of Community mineral horizon dormant growing bacteria – according to seeding of casein and starch as possible *Bacillus*.

Conclusions

Studies of bacterial communities in the samples of Antarctic soils by different methods showed that, both in liquid soil suspensions and *in situ*, microbial complexes are functioning presumably by forming biofilms – the phenomenon that is more expressed in such a habitat than in soils of temperate zones.

The recent study of soil-forming processes in the oases of East Antarctica, where soil samples were taken for microbiological examination, revealed that the organic-film may be the only product of soil formation in the absence of "classical" soil horizons (Mergelov et al., 2014). Such films can also protect cells from adverse environmental impacts as well as regulate their physiological state at the expense of communication links within the

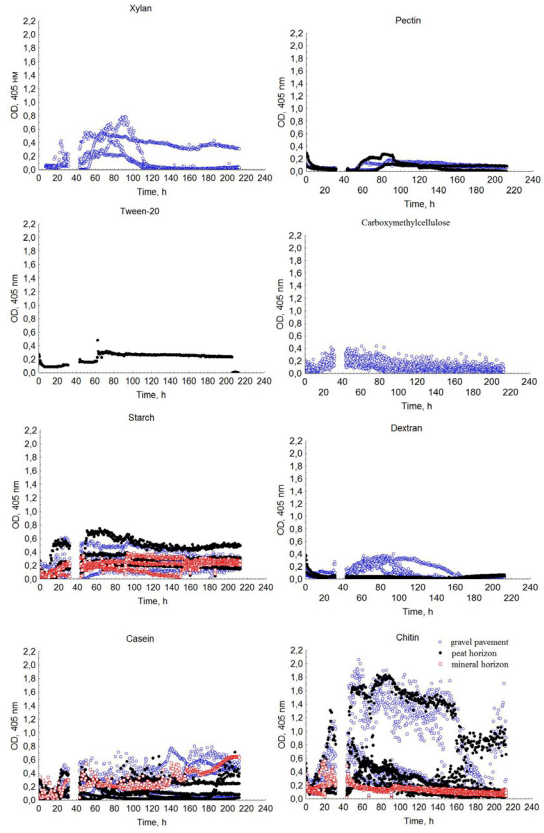

Figure 7: The growth in a mineral medium with different biopolymers.

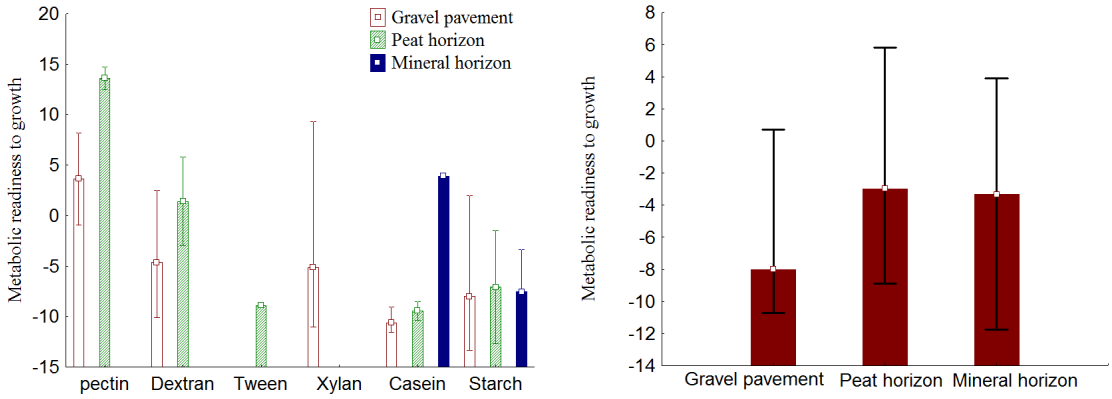

Figure 8: Metabolic preparedness growth on polymers of microbial associations.

community immersed in a matrix of the biofilm. It can be assumed that "brown crusts" or biofilms in Antarctic soils, due to their withstanding to extreme conditions, may be not destroyed. Thus, it can make difficult the desorption process of the cells in the application of standard methods for the isolation of bacteria from such substrates. The latter requires

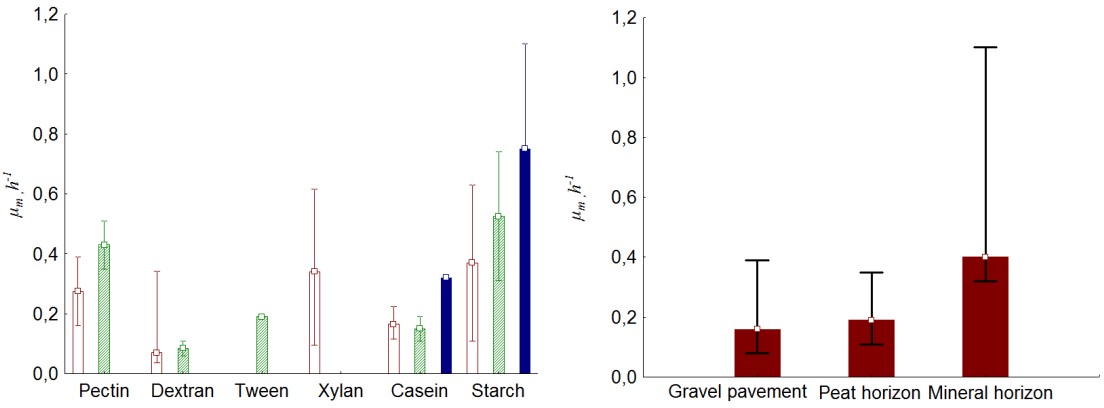

Figure 9: The maximum specific growth rate μ_{m} bacterial associations in the liquid media with polymers.

further improvement techniques of isolation and stimulates growth procedures on nutrient media because of different physiological state in biofilms.

The investigated samples of Antarctic soils with gravel pavement, peaty and mineral horizons revealed different trophic diversity and the maximum specific growth rate on mineral medium with different biopolymers as the sole carbon source. It should be noted that a higher functional diversity was detected in upper horizon of gravel pavement, which "protects" microorganisms from exposure to extreme temperatures, UV radiation, and desiccation. However, the maximum specific growth rate was higher in the lower mineral horizon in spite of lower number of CFU/g in comparison with upper layers. This can be explained by the specificity of bacterial colonizing processes and unique formation of soil microprofiles in the Larsemann oasis, which distinguishes such soils from soils of temperate latitudes.

It was shown that microbial communities that formed micro profiles of investigated Antarctic soil were characterized by functional (tropic) diversity and the rate of growth on nutrient media with different carbon sources.

The latter may suggest differences in the initial physiological state of the bacterial populations in various soil horizons. Antarctic primitive soils, forming on the surface of the rocks, seem to be not less attractive as a model for study of life preservation than Antarctic permafrost. In contrast to subsurface layers they are characterized by less stable external factors, due to changing cycles of freezing and thawing and high doses of UV radiation that make such biotopes more extreme for microbial survival.

In spite of the harsh conditions and lack of higher plants, bacterial communities are able to colonize the subsurface layers of Antarctic species and maintain their metabolic activity in biofilms forming soil microprofiles, as evidenced by our findings about the differences in the utilization of various substrates by hydrolytic bacteria.

References

1. Добровольская Т.Г., Головченко А.В., Кухаренко О. С., Якушев А. В., Семёнова Т.А., Инишева Л. И. Структура микробных сообществ верховых и низинных торфяников Томской области // Почвоведение, 2012, № 3, 317–326.

2. Якушев А.В., Кухаренко О.С. Кинетический метод определения физиологического состояния бактерий *in situ* / Гл. в монографии "Функционирование микробных комплексов в верховых торфяниках – анализ причин медленной деструкции торфа". Москва: Товарищество научных изданий КМК. 2013. С 49–55.

3. Якушев А.В. Микробиологическая характеристика вермикомпостов. Автореферат дис. ... канд. биол. наук. М., 2009. 24 с.

4. Методы почвенной микробиологии и биохимии / Под ред. Д.Г. Звягинцева. М.: Изд-во Моск. ун-та, 1991. 303 с.

5.Vishniac H.S. The microbiology of Antarctic soils. // In: E.J. Friedmann (ed.) Antarctic Microbiology, Wiley-Liss, Inc. 1993, P. 297.

6. Yergeau E., Newsham K.K., Pearce D.A., and Kowaichuk G.A. Patterns of bacterial diversity across a range of Antarctic terrestrial habitats // Environmental Microbiology **9**, 2670–2676 (2007).

7. Mergelov N.S. Soils of wet valleys in Larsemann and Vestfoll oasis (Princess Elizabeth Land, East Antarctida) // Soil Science, 2014, #9, 1–19.

Исследование функционирования бактериальных комплексов в почвах восточной Антарктиды как модель для астробиологии

А.В. Якушев[*], Н.А. Чурилин[*], В.С. Соина[*], А.Г. Кудинова[*]
Е.А. Воробьёва[*,], Н.С. Мергелов[***]**

[*] *Факультет почвоведения Московского государственного университета имени М.В. Ломоносова*
Воробьёвы Горы, Москва, 119234 Россия
E-mail: a_ yakushev84@mail.ru

[**] *Институт космических исследований РАН*
Профсоюзная ул. 84/32, Москва, 119997 Россия

[***] *Институт географии РАН*
Старомонетный пер. 29, Москва, 109017 Россия

Резюме. Исследование бактериального сообщества в образцах почв Антарктиды различными методами показало, что как в жидких суспензии почвы, так и *in situ* микробные комплексы функционируют, по-видимому, образуя биоплёнки – явление, которое более присуще таким местообитаниям, чем почвам умеренных зон. Функциональное (трофическое) разнообразие и физиологическое состояние гидролитических бактерий в образцах верхнего горизонта (0–2 см) – каменная мостовая с водорослями, торфяного горизонта (2–4 см) с включениями мёртвой биомассы и подстилающего минерального горизонта (4–10 см) с признаками грибного мицелия. Исследуемые образцы антарктических грунтов показали различное трофическое разнообразие и максимальную удельную скорость роста на минеральной среде с различными биополимерами в качестве единственного источника углерода (крахмал, хитин, пектин, ксилана, декстран-500, твин-20, казеин), что может указывать на различия в физиологическом состоянии гидролитических бактерий в различных почвенных горизонтах и в готовности их к росту. Наиболее значимые характеристики изучаемой антарктической почвы по сравнению с почвами умеренного пояса – необычная способность гидролитического сообщества потреблять хитин в минеральном горизонте, что можно объяснить наличием грибного мицелия. Кроме того, почти не потребляется Твин-20 (водорастворимый аналог жира) бактериальным сообществом арктических почвенных горизонтов. Этот факт необъясним и нуждается в дальнейшей проверке. Наибольшее функциональное разнообразие было обнаружено в верхнем горизонте каменной мостовой, которая "защищает" микроорганизмы от воздействия экстремальных температур, ультрафиолетового излучения и высыхания, а максимальная удельная скорость роста была выше в нижней минеральном горизонте, что может быть объяснено спецификой бактериальных процессов колонизации и уникальностью формирования микропрофиля антарктических почв в оазисе Ларсеманн. Полученные данные указывают на конкретную экологическую стратегию в образцах почв Антарктики: развитие в нижнем минеральных горизонтах микроорганизмов с высокой метаболической готовностью к росту и высокой максимальной скоростью роста.

Работа поддержана грантом РФФИ № 13-04-01982-а, а также Программой фундаментальных исследований № 28 Президиума РАН "Проблемы происхождения жизни и становления биосферы"; подпрограмма 2; направление 1. Предпосылки возникновения жизни в Солнечной системе и за её пределами.