ANALYSIS OF SOIL MICROBIOME INDICATORS IN PROCESSES OF SOIL FORMATION, ORGANIC MATTER TRANSFORMATION AND PROCESSES INVOLVED WITH FINE REGULATION OF VEGETATIVE PROCESSES

E. E. Andronov[1], E. A. Ivanova[2], E. V. Pershina[1], O. V. Orlova[1], Yu. V. Kruglov[1], A. A. Belimov[1], I. A. Tikhonovich[1]

[1] All-Russian research institute for agricultural microbiology, 196608,
St. Petersburg, Pushkin, 8, highway Podbelskogo 3
[2]V.V. Dokuchaev Soil Science Institute, 119017 Moscow, Pyzhevskii 7, bld.2
e-mail: microbioliza@gmail.com

At the current stage of soil microbiology development the study of the entire complex of microorganisms inhabiting the soil became possible, which helps finding the optimal combination of factors resulting in the formation of soil fertility as well as the development of stable and stress resistant phytocenosis. Soil metagenome is the largest genetic depository for all purposes, from the soil formation processes – transformation of barren rock into a substrate for plant growth and development, to the temporary adaptations in the short-term interests of the plant. The investigation of genetic potential of soil metagenome and its mobilization are the main goals of this project. Such studies should be comprehensive and solve actual issues: 1) the study of the mechanisms of soil-forming processes and the analysis of the evolution of metagenomes due to the special features of pedogenesis; 2) investigation of microbiome participation in organic residues decomposition and efficient transformation of soil organic matter; 3) screening of the associative plant-protective and growth promoting microorganisms. The object for the analysis of microbiome evolutionary potential in the process of soil formation may be the technogenic dumps resulting from mining operations, which are chronosequences of soils of different ages. Samples of paleosois are also of particular interest in the evolutionary aspect of the analysis of the soil metagenome. The project is expected to reveal a group of soil microorganisms, which take the most active part in the formation of soil fertility and effectively implement growth-stimulating and protective functions for the plant. The data may be of value for both fundamental science and

serve as a base for the design of environmentally friendly high-productive phytocenoses based on the use of the adaptive potential of soil microbiota.

Key words: soil metagenome, high-throughput sequencing, genetic potential, evolution.

Today, one of the key problems in soil science is the biosphere evolution including the soil genesis attributed to the evolution of soil microbiomes. Just soil microorganisms perform the substrate-forming functions in such processes as the soil formation, the organic matter decomposition in soil, the growth stimulation of plants and their protection from phatogenic microflora [11, 16]. The study of these processes in the context with the soil metagenomes seems promising for a better understanding of the soil cover evolution and the formation of the soil fertility. In view of this, the present studies are aimed at discovering and mobilizing the genetic potential of the soil microbiome. Being conducted within the framework of the project these studies include the stages coinciding with those inherent to the soil development (metagenome): the soil formation, humus accumulation resulted from decomposition of the organic matter in soil as well as the formation of associative microbiomes capable to increase the adaptive potential of plants. Of great interest are also the samples of paleosoils that have been developed more than 100 years ago. It will help to answer the question whether can serve these soil samples as genetic information on the microorganisms inhabited the given soil or the irreversible succession of microbial communities took place for a long period of time. Besides, the microorganisms identified in samples of soils developed 100 years ago may be very interesting and promising from the viewpoint of studying the dynamics of the soil microbiome in the range of this time scale. The distinctive peculiarity of the given study is its multidisciplinary and complicated character combined the traditional problems of evolution in soil science with the latest achievements in the study of metagenomes. The plan of the present studies is rather peculiar because a comprehensive analysis of literature sources as well as the experience gained in the course of the authors' participation in special international conferences permit to indicate that the problem of soil genesis in the light of modern studies of metagenomes has been so far examined insufficiently.

APPLICATION OF METAGENOMIC APPROACHES IN FUNDAMENTAL AND PRACTICAL STUDIES OF THE SOIL GENESIS AND EVOLUTION OF THE SOIL MICROBIOTA EXEMPLIFIED BY CHRONOSEQUENCES OF ROCK DUMPS

A classical but still actual model for the succession of microbial communities in the course of the soil formation is technogenically transformed landscapes and different-aged rock dumps in particular to be a series of soil chronosequences [1]. In the fundamental science such systems are an object for elaborating ecological models of the pedogenesis determining not only by the vegetation in disturbed agrolandscapes but also by evolution of the soil microbiome as one of the substrate-forming factors in soil ecosystems. The study of soil restoration processes will be a valuable contribution to the development of the conception in genetic soil science. The study of earlier stages of pedogenesis (to 100 years) indicating the peculiar soil development in dependence on the composition of parent rocks is of great importance. The role of soil microbiome in the given process and the use of its potential as a universal ecological indicator will allow identifying the soil microorganisms and peculiar features of the soil formation. The study of rock dumps resulted from mining operations is also of great practical importance because the technogenic contamination of the environment is a factor determining destabilization of natural ecosystems nowadays [3, 7]. As a result of these processes the potential fertile lands become degraded; today more than 40% of their area is suffered from such adverse effects. In the light of accelerating the degradation of agriculturally valuable lands and those included into the ecological stock it is very important to elaborate approaches designed to restore the disturbed ecosystems into the regime of natural functioning (their rehabilitation). In the world practice of recreation the regenerated possibilities of proper natural ecosystems are used and the great role is played by such biotic factors of the soil-forming process as the development of the vegetation cover associated with soil microbiome, the latter being underestimated in the soil genesis for a long period of time. The microorganisms are capable to utilize a wide spectrum of chemical compounds, thus participating in bioremediation of disturbed areas. Besides, the microbial community is one of the most sensitive ecological indicators for different stages in soil restoration because it enables to adapt to different changes in their habitat and occupy all the available ecological niches in the ecosystem.

The first stage in these studies is the inventory of rock dumps at the territory of Russia. To select such an object of research it is necessary to have a series of different-aged rock dumps demonstrating different stages in the soil formation process. In dumps of different age it is planned to describe the profiles and to take soil samples from every horizon for their further studying in the analytical laboratory. The age of some dumps is not always dated exactly; by this reasonit is assumed to use a definite agrochemical marker represented by the percentage of humus carbon; its value will be characteristic of the old age for the topsoil in zonal soil that hasn't been subjected to technogenic effects.

The main objective of these studies can be realized by analyzing the taxonomic structure of microbiomes in the studied soil samples according to data about the high-productive sequencing of profiles in soils under recultivation and the identification of relationships between the peculiar features of the soil microbiome, symgenetic succession of plants and the dynamics of soil characteristics. The study includes the total DNA and RNA extracted from all the soil samples and the quantitative analysis of archean, bacterial and fungi microbiota as well as the sequencing of taxonomically valuable parts of genome (16SpPHK, ITS). The agrochemical analysis of the studied samples includes the analysis of the following indices for the agro-environmental status of soil: the content of different organic matter forms, pH in water and salt extracts, ammonium and nitrate forms of nitrogen, available phosphorus and potassium, exchangeable calcium and sodium; the particle size and aggregate analyses will be also made. With the view of identifying a plant component it is necessary to give a geobotanical characteristic of plant phytocenoses at different stages of soil recultivation. The given stage of the project will permit to study the dynamics of microbial succession in the soil formation process and soil restoration in technogenic landscapes as well as identification of microbial taxa promising from the viewpoints of improving the soil-environmental indices; they can be also used for creating the microbial preparations to increase the quality of biological recultivation.

ANALYSIS OF MICROBIOMES IN SOIL SAMPLES FROM THE MUSEUM

One of the stages in the studies within the framework of this project is the analysis of microbiocenoses in soil samples taken in museum of the V.V. Dokuchaev Soil Science Institute. The given studies are of great scientific interest in the light of comprehending the evolution of the soil cover. By analogy with the DNA extracted from archaeological findings of human remains they will allow empirical testing of evolutional hypotheses and obtaining the information on “genetic peculiarities” of soils existed more than 100 years ago. Of special interest are soil samples that have been taken in fixed places, in which the samples of the recent soil can be taken now. The required stages in this study are the DNA extraction from soil samples, the quantitative analysis of archaen, bacterial and fungi microbiota as well as sequencing of taxonomically valuable parts of the genome (16SpPHK, ITS). Under analysis will be the microbiome DNA extracted from the museum soil samples for comparing with those in samples taken in the recent soil.

In a set of vegetation experiments it is planned to identify the useful symbiotic and associative microorganisms inhabiting the museum soil samples. In case of success the peculiar structure of the genome so vital for fertile properties of stocks will be studied in the given microorganisms.

EXPERIMENTS TO STUDY THE MICROBIOLOGICAL DECOMPOSITION OF THE ORGANIC MATTER IN SOILS OF DIFFERENT GENESIS

The important moment in the soil formation process is the development of the soil fertility thanks to the humus accumulation resulted from the organic matter decomposition of plant remnants. The rate of destruction processes and transformation of the organic matter in soil is a key problem for forecasting the state of terrestrial ecosystems. The soil microorganisms play an essential role in the humus formation [5, 11]. This is evidenced by the temperature curve of humification identical to the curve of fermentative reactions. Besides, the microorganisms taking part in mineralization processes form the dark humus-like compounds – melanins, the structure of which is identical to humic acids [4, 14].

By this reason, one of the most important goals is the study of biogeochemical processes of formation, transformation and accumulation of the organic matter affecting by microorganisms. The widely adopted method to study the transformation of the organic matter in plant residues is modeling of this process by isolated probing in field and laboratory [8, 16]. However, in field there is no possibility to detect the influence of definite factors; occasional or systematic errors can be happened. In laboratory the experiment is under control and it is feasible to take into consideration the changes in the substrate. In view of this, it is planned to carry out model laboratory experiments to study the microbiological process of the organic matter decomposition in soils of different genesis.

One of the ways for studying the above process is the study of taxonomic structure of microbiomes in the soil samples by using taxonomically valuable markers (16 SpPHK and ITS fragments) at different stages of the organic matter decomposition. Promising is the analysis of the taxonomic profile of general microbiological picture based upon the analysis of total DNA as well as the expressional profiles of microbiome. As a substratum the cellulose and rye straw power-like samples can be used. For this reason it is necessary to study the dynamics of functional activity of the cellulose isolated complex of microorganisms in different soils (chernozems and soddy-podzolic soils), thus analyzing the succession of expressive gene profilesbearing a relation to the decomposition process of the organic matter in soil. The necessary stage in the given analysis is constructing the primers for genes responsible for destruction and transformation of carbon in soils. The obtained data can be served as a base for creating predicative models of the dynamics of microbiomes in the course of the organic matter decomposition in dependence on the decomposed substance type and peculiar physical-chemical properties of soils. Thanks to cultivation and the use of the up-to-date methods of molecular biology the groups of microorganisms will be detected so perspective from viewpoints of creating microbiological preparations.

Byulleten Pochvennogo instituta im. V.V. Dokuchaeva. 2015. Vol. 80.

EXPERIMENTS TO STUDY SCREENING OF SOIL METAGENOMES WITH THE VIEW OF INCREASING THE STABILITY AND STIMULATION OF THE PLANT GROWTH AND DEVELOPMENT BY ANALYZING THE RIZOSPHERIC MICROBIOMES

Microorganisms should be considered as a link in the soil-plant system providing the plant growth and development as well as the ecological adaptation of plants to stress situations. For today the actual task is the search of pathways for using the resources of the plant-microbial systems in order to maintain the high productivity of plants, the soil fertility and to decrease the adverse effect of stress-induced factors on phytocenoses.

The rhizosphere is a soil zone adjacent to the root system and a basic ecological niche for rhizobacteria, in which there are favorable conditions for their existence. The rhizospheric and endophytic microorganisms play an essential role in the plant vitality, thus promoting the uptake of nutrient elements regulating the hormonal balance for direct or indirect protection of plants from harmful organisms (for instance, pathogenic ones) and abiotic stresses (dewatering, adverse effects of heavy metals) as well as improving the soil structure.

At the present time, there are a number of mechanisms responsible for the positive influence of rhizobacteria on the plant vitality [6]. One of such mechanisms of interaction between the plant and bacterial associations is producing of phytohormones by bacteria, vitamins and the other biologically active substances. Special attention is paid to the role of bacterial auxins because their capacity to synthesize the phytohormon of indole-3-acetic acid is widely distributed among rhizobacteria [9, 13]. It is known that the synthesis of auxins by rhizospheric microorganisms is determined by the composition of root excretion containing the metabolic L-tryptophane [13]. However, the experiments are scanty to study the changes in the content of hormones in plants affecting by the growth-promoting bacteria and the ability of bacteria to destroy phytohormones [9]. The bacteria producing the ferment of 1-aminocyclopropan-1-carboxilate (ACC) diaminase posses a universal anti-stress action to decline the ethylene content in plants – a signal molecule with varying cascade of unspecific stress and adaptive reactions [9, 10]. The ACC amino acid is preceding the biosynthesis of ethylene phytohormone being oxidized by ACC ferment oxidase to the ethylene with the formation of CO2, cyanic acid and water [15]. The ethylene involves into many stages of the growth and development of plants including the seed germination, initiation of plant tissues and organs, flowering, fruit maturation and reactions on stress-induced factors. The activity of ACC and ethylene biosynthesis is an unspecific reaction of plant on different stresses.

Thus, the goal of the given stage in the studies within the framework of the present project is the search of associative microorganisms providing the protection of plants and stimulation of their growth to be a basis for the formation of the valuable agrophytocenosis resistant to different stresses. In view of this, it is necessary to conduct a vegetation experiment to grow two contrasting crops (for instance, wheat and rye) on two contrasting soil types (soddy-pozolic and chernozem). The following procedures are extraction of total DNA and RNA from the rhizosphere of crops, the analysis of rhizospheric microbiome and its metabolitically active part by using taxonomically valuable markers (16S and ITS) and screening of microorganisms – producers of phytohormones (auxins, ACC-desaminase) performed by the colorimetric method. With the help of universal primers for genes producing auxins and ACC-desaminase the diversity of genes responsible for the hormone biosynthesis will be comprehensively analyzed in the extracted DNA and cultivated forms of microorganisms in order to identify a share of the cultivated compound in total community of auxin-and ACC-deaminase producing the microorganisms inhabiting the soil. It is necessary to determine their concentration in the studied communities by Real-TimePCR method. As a final stage it is foreseen to identify perspective microorganisms – producers as potential components of microbial preparations for accelerating the growth and development of plants on different substrates.

CONCLUSION

As a result of the given project it is assumed to detect the groups of soil microorganisms that take an active part in the formation of the soil fertility and perform the growth-promoting and plant-protecting functions. The data obtained in the course of the system analysis of relationships of the structure and dynamics of microbial communities with peculiarities of syngenetic succession of vegetation and soil agrochemical and agrophysical characteristics will be used for constructing new predicative models of microbiological succession processes. These models can be a base for ecological and monitoring soil studies as well as technogenically transformed landscapes. The novelty of the project is the application of metagenomic approach to studying the soil genesis. As far as the soil genesis is conditioned by physiological activity of the soil microbiota, its study is of principal value for a better understanding the mechanisms responsible for the soil formation processes.

REFERENCES

1. Abakumov E.V., Gagarina E.I., Lisitsyna O.V. Restoration of soils and land reclamation in the area Kingisepp deposit phospho-ferrites, *Eurasian Soil Science*, 2005, No. 6, pp. 731–740.
2. Belimov A.A., Safronova V.I. ACC deaminase and plant-microbe interactions, *Agricultural Biology*, 2011, No. 3, pp. 23–28.
3. Kornilov I.A., Novykh L.L., Kornilov A.G. Geoecological situation in the industrial area of the Belgorod region, *Geology, geography and global energy*, 2012, Vol. 45 (2), pp. 221–227.
4. Orlov D.S., Biryukova O.N., Sukhanova N.I. *Soil organic matter of the Russian Federation*, Moscow, 1996, 253 p.
5. Fedotov G.N., Lysak L.V. The possible role of microorganisms in an education-humic substances in soils, *Dokl. Russian Academy of Sciences*, 2014, T. 455(1), pp. 114–117.
6. Shaposhnikov A.I., Belimov A.A., Kravchenko L.V., Vivanko D.M. Interacteraction rhizosphere bacteria with plants: the mechanisms of education, and factors of efficiency of associative symbioses, *Agritural Biology*, 2011, No. 3, pp. 16–22.
7. Yabrova Yu.I., Shvabenland I.S. The role of soil biota to soil formation initial overburden dumps East Beysky coal-foot section of the republic of Khakassia, *Bulletin of the Volga VN universitetata name Tatishcheva*, 2012, No 3.
8. Balamurugan A., Jayanthi R., Nepolean P., Pallavi R.V., Premkumar R. Studies on cellulose degrading bacteria in tea garden soils, *African J. Plant Sci.*, 2011, Vol. 5(1), pp. 22–27.
9. Belimov A.A., Hontzeas N., Safronova V.I. et al. Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (Brassica juncea L. Czern.), *Soil Biol. Biochem.*, 2005, Vol. 37, pp. 241–250.
10. Czarny J.C., Grichko V.P., Glick B.R. Genetic modulation of ethylene biosynthesis and signaling in plants, *Biotechnol. Adv.*, 2006, Vol. 24, pp. 410–419.

11.Condron L., Stark C., O'Callaghan M., Clinton P., Huang Z. The Role of Microbial Communities in the Formation and Decomposition of Soil Organic Matter, *Soil Microbiology and Sustainable Crop Production*. Springer Science+Business Media B.V., 2010, pp. 81–117.

12.Eichorst S.A., Kuske C.R. Identification of Cellulose-Responsive Bacterial and Fungal Communities in Geographically and Edaphically Different Soils by Using Stable Isotope Probing, *Appl. Environ. Microbiol*., 2012, Vol. 78, № 7, pp. 2316–2327.

13.Frankenberger W.T. Arshad M. *Phytohormones in soils: production and function.* Marcel Dekker, Inc. N.Y., 1995, 503 p.

14.Gomes R.C., Mangrich A.S., Coelho R.R., Linhares L.F., Gomes R.S. Elemental, functional-group and infrared spectroscopic analysis of actinomycete melanins from Brazilian soils, *Biol. Fert. Soils*, 1996, Vol. 21(1–2), pp. 84–88.

15.Nascimento F.X., Rossi R.M.J., Soares C.R.F.S., McConkey B.J., Glick B.R. New Insights into 1-Aminocyclopropane-1-Carboxylate (ACC) Deaminase Phylogeny, Evolution and Ecological Significance, *PLoS ONE*, 2014, Vol. 9(6), doi: 10.1371/journal.pone.0099168.

16.Schulz S., Brankatschk R., Dumig A., Kogel-Knabner I., Schloter M., Zeyer J. The role of microorganisms at different stages of ecosystem development for soil formation, *Biogeoscience*s, 2013, Vol. 10, pp. 3983–3996.