AGRICULTURAL BIOLOGY,
2016, V. 51, № 5, pp. 654-663
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

Plant and soil

UDC 573.22:581.55

doi: 10.15389/agrobiology.2016.5.654rus
doi: 10.15389/agrobiology.2016.5.654eng

METAGENOMIC CHARACTERISTIC OF RHIZOSPHERE EFFECT ON CEREALS IN BLACK AND SOD-PODZOLIC SOILS

A.O. ZVEREV, E.V. PERSHINA, N.A. PROVOROV, E.E. ANDRONOV, E.N. SERIKOVA

All-Russian Research Institute for Agricultural Microbiology, Federal Agency of Scientific Organizations, 3, sh. Podbel'skogo, St. Petersburg, 196608 Russia, e-mail azver.bio@gmail.com
Acknowledgements:
Supported by Russian Science Foundation (project 14-26-00094)
Received July 6, 2016

Abstract

Changes in the composition of microbial communities under the influence of root exudation of plants (rhizosphere effect) is widely reported in the scientific literature. A number of studies clearly show the rhizosphere effect of external factors such as soil type, species and plant variety, etc. The aim of this work is to study the effect of soil type and plant species using modern high-throughput sequencing techniques. This effect has been studied well by foreign counterparts, but such work on Russian soils and crops used in the domestic agro-industry, is carried out for the first time. We used two soils contrasting by their agrochemical parameters, black earth (Voronezh region), and sod-podzolic soil (Pskov region). Rye (*Secale cereale* L., k-6469) and wheat (*Triticum aestivum* L., k-54609**)** seeds obtained from VIR collection (St. Petersburg) were grown in a greenhouse on both soils for 42 days. Using NGS-V4 variable region sequenced 16S rDNA gene, microbial community composition in bulk soils and the rhizospheres formed on them was analyzed. Despite the short period of the experiment, clear rhizosphere effect was revealed in both soils. The strongest factor was the type of soil. Communities of bulk soil as well as rhizosphere communities on these soils, were significantly different from each other. Both soils show the same effect in the formation of rhizosphere communities of rye and wheat. Type of plant is the second largest (after the type of soil) factor in determining taxonomic composition of the rhizosphere microbiome. Communities of rye rhizosphere in general are closer to the communities of bulk soils than wheat rhizosphere communities. Also, the rhizosphere communities of rye on sod-podzolic soil according to the cluster analysis are closer in structure to the original communities of the soil. The taxonomic analysis of the communities at the level of phyla revealed several groups. They are most responsible for the rhizosphere effect. Formation of rhizosphere communities was accompanied by an increase in the number of *Betaproteobacteria* class sequences, while reducing the part of the bacteria of *Verrucomicrobia* phylum. Significant changes in the community occurred in wheat-cultivated sod-podzolic soil. According to the results of all analyzes, these communities differ significantly from the original communities of soil and rhizosphere communities of rye on sod-podzolic soil. Perhaps this can be attributed to an increased proportion of the genus *Flavobacterium* (phylum *Bacteroidetes*) bacteria in these communities. Using the method of high-throughput sequencing it has been clearly demonstrated the presence of rhizosphere effect on rye- and wheat-cultivated soils, as well as the features of the interaction of individual factors responsible for rhizosphere effect. However, to confirm rhizosphere effect, as well as for more detailed studies of the mechanisms underlying it, it is necessary, in addition to the taxonomic analysis carried out, to elucidate how the rhizosphere microbiome is influenced by the plant exudate composition. To do this a series of model experiments with introduction into the soil of certain root exudate substances of rye and wheat are already scheduled.

Keywords: rhizosphere effect, rhizosphere microbiom, metagenomic analysis, rye rhizosphere, wheat rhizosphere

The rhizosphere of plants represents a special niche, where microbial community specific for each species of plant is formed [1-4]. The structure of this community is largely determined by the composition of plant exudates, performing both the role of the substrate and regulatory functions [3-6]. Owing to the exudation process, a plant actively cooperates with the soil microbiota, forming the microbial environment which provides the plant with a number of adap-

tive advantages, such as protection from pathogens, mineral nutrition, adaptation to abiotic stresses, and regulation of the development [3-6]. The development of a plant, the growth of its roots and root exudation is a powerful biotic factor contributing to the formation of rhizosphere microbiome [6-9]. Qualitative and quantitative modification in the composition of microbial community under its influence became known as the rhizosphere effect [6, 8]. It was shown that it manifested differently in different soils [10, 11], at different stages of plant development [12], and in different plant species and even varieties [10-14]. Significant differences in the rhizosphere effect were also identified when cultivated plants were compared with initial wild relatives [14, 15].

For a long time, the rhizosphere effect was studied using conventional microbiological methods, which thereby provided a wealth of scientific experience with respect to both physiological and genetic properties of the main representatives of the rhizosphere microbiome [2, 6, 8]. However, as it is known, only a small part of the diversity was covered in these studies [16, 17]. Modern molecular techniques, such as high-throughput sequencing, made possible a more detailed examination of the rhizosphere microbiome, including not only cultivated, but also its uncultivated representatives. This method is widely used by foreign scientists [18-22], but in the domestic soil investigations there is obviously a lack of such research. The use of high-throughput sequencing when investigating the rhizosphere effect allowed to show conclusively the role of the soil type, the duration of growth and plant variety [10, 12-14] in determining the taxonomic composition of the rhizosphere microbiomes. All the studies indicated that it was the type of soil, which had the greatest influence on the rhizosphere effect [10, 13, 22]. The rhizosphere effect was examined in a number of plants, i.e. from model objects, such as *Arabidopsis* sp. [17, 18, 21], to the species of major agricultural importance, e.g. rice (*Oryza sativa*) [23], and lettuce (*Lactuca sativa*) (22). In these cases, the rhizosphere effect manifested differently for different plants. It should also be noted that in most reports the rhizosphere effect was studied for soil types which were similar in structure and genesis [11, 17, 21].

Considering that the rhizosphere effect manifests itself depending on the characteristics of plants and soil types, our primary objective was to expand the circle of diversity of the tested subjects. This is the first paper to report a study of the rhizosphere effect for the agricultural crops common in Russia (rye and wheat varieties), and in commonly occurring contrastive soils (chernozem and sod-podzolic).

The objectives of the study included the evaluation of the rhizosphere effect in model experiments on the cultivation of rye and wheat plants in soils of different types, with concurrent identification of specific taxonomic groups of bacteria.

Technique. The soil samples for the experiments were taken in the agriculturally used areas (division edges on the fields free of crops over the last 50 years) at a depth of 3-15 cm. Sod-podzolic soil samples were provided by Pskov Agricultural Research Institute and the state owned farm «Rodina» (Pskov Province, the coordinates of the sampling point: 57°50'44,2"N, 28°12'03,7"E).) Chernozem samples were obtained from Voronezh Province (the nature preserve Kamennaya Steppe: 510°01'41,6"N, 400°43' 39,3"E). The soil samples were sieved on a 5 mm soil screen, dried and filled into plastic containers (by 5.0 kg for chernozem and 5.5 kg for sod-podzolic soil) and then humidified at the rate of 75 % of total moisture capacity.

One day after, seeds were introduced in each pot to a depth of 3-5 cm in regular rows, 25 pcs per pot. We used rye seeds of a local variety seeded only in Pskov Province (k-6469 in the VIR catalog — N.I. Vavilov All-Russian Institute

of Plant Genetic Resources, St. Petersburg), and wheat seeds (the Volshebnitsa variety, k-54609 in the VIR catalog). Two pots with each type of soil were used per each variety. The experiment was carried out for 42 days (from September 23 to November 4, 2014) in the greenhouse covered with a plastic wrap (the end wall was covered with a mesh to provide gas exchange), while maintaining a constant soil moisture (75 % of the total moisture capacity). The average daily temperature during the experiment was 13 °C and nighttime temperature 4 °C. At the end of the experiment, two samples of roots were taken from each pot. The roots, as soon as separated from the soil, were divided into two roughly equal portions, then placed in vials with water (50 ml) and shaken vigorously for 1 minute to obtain a homogeneous suspension of soil. A 2-ml aliquot of the suspension was collected into a microtube, centrifuged, and the pellet was used to isolate the rhizosphere DNA.

DNA was isolated using the method developed in the All-Russian Research Institute for Agricultural Microbiology [25]. The resulting DNA concentration was on average 18 ng/ml. The purified DNA was used as a template for the PCR with universal primers targeting variable region 4 of the 16S rRNA gene, the F515 GTGCCAGCMGCCGCGGTAA and R806 GGACTACVSGGGTATCTAAT [26], with the addition of the oligonucleotide identifiers for each sample and supporting sequences required for pyrosequencing technology. NGS-sequencing (next-generation sequencing) was carried out using a GS Junior system (Roche, USA) according to the manufacturer's recommendations.

The data were processed in QIIME, v.1.8.0 (http://qiime.org/) [27]. The sequences of the 16S rRNA gene were analyzed in several stages. The first stage involved quality control of the sequences to exclude from the analysis those with length less than 200 nucleotides, with a quality score of less than 25, with misread sequences of primers and multiplex identifiers, extensive homopolymer repeats (more than 8 nucleotides) and unidentified nucleotides. After excluding all non-bacterial and chimeric sequences, the resulting libraries were normalized according to the number of sequences in the smallest library. As a result of all the procedures performed, 19,440 sequences were selected (810 in each library). The sequences with a > 97 % similarity were combined into operational taxonomic units (OTUs), using the de novo algorithm (based on the «uclust» method). One sequence was selected from each OTU to produce a set of representative sequences. The next stage was the classification of representative sequences using the RDP naïve Bayesianr RNA Classifier, and the alignment using the PyNast algorithm [27], where a specially designed Greengenes coreset of sequences served as a matrix for alignment [28]. After aligning, the sequences were used to construct gene distance matrix and the phylogenetic tree.

To characterize biodiversity and carry out a comparative analysis of the communities, the parameters of α- and β-diversity were calculated. The α-diversity was assessed using species richness indices (the OTU value in the sample) and the Shannon index (Shannon, H). The significance of differences in the α-diversity indices between the microbiomes was determined using *t*-test. To assess β-diversity the Weighted unifrac method was used, allowing to identify the percentage of similarities between all pairs of the microbiomes being compared [29]. The results were presented using methods of the PCoA multivariate statistics (principal component analysis) and data were visualized in the Emperor program (is a part of QIIME) (http://emperor.colorado.edu). For calculating the indices of diversity and performing the cluster analysis, the Bray-Curtiss criterion was used and calculations were carried out in the PAST software (http://folk.uio.no/ohammer/past/) [30]. Statistical support for clusters was calculated via the bootstrap method (1,000 replacements).

The differences between the samples in terms of the taxa frequency were determined using the Fisher's exact test adjusted for multiple comparisons by the Benjamini-Hocberg FDR procedure at the 5 % significance level.

Results. We used primers which were designed based on the analysis of nucleotide sequences of both bacteria and archaea, and allow to amplify the 16S rRNA gene fragment of approximately 400 bps. The paper analyzed the microbiome communities in six variants, such as the sod-podzolic soil (SP), chernozem (ChZ), the rye rhizosphere in the sod-podzolic soil (rSP), the wheat rhizosphere in the sod-podzolic soil (wSP), the rye rhizosphere in the chernozem (rChZ) and the wheat rhizosphere in the chernozem (wChZ).

Indices of diversity. Indices of diversity calculated for soil communities and rhizosphere communities were not significantly different. Significant differences in the values of the Chao-1 and Shannon indices were demonstrated for the community of wheat rhizosphere in the sod-podzolic soil (see Table). As can be seen, the values of both indices were significantly lower than in communities of the sod-podzolic soil or the rye rhizosphere on the same soil.

Indices of α-diversity for soil and rhizosphere microbiome communities, depending on the soil type and plant species ($X \pm x$, wheat *Triticum aestivum* L. and rye *Secale cereale* L.)

Microbiome	Indices of diversity		
	Species richness	Chao-1	Shannon (H)
Chernozem (Voronezh Province)			
No plants	277±35	360±41	4.94±0.17
Rhizosphere:			
of wheat	219±21	310±31	4.78±0.13
of rye	297±23	426±18	4.96±0.04
Sod-podzolic soil (Pskov Province)			
No plants	287±33	401±28	5.20±0.11
Rhizosphere:			
of wheat	248±26	335±31	4.78±0.07
of rye	290±22	393±10	5.09±0.05

Note. Rye (k-6469 in the VIR catalog, N.I. Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg), a local variety from Pskov oblast) and wheat varieties (the Volshebnitsa variety, k-54609 in the VIR catalog) were used.

The observed effect is of particular interest in relation with the available literature data, i.e. previous reports indicated that indices of diversity of rhizosphere communities were not significantly different from those of the initial soil communities both in case of different soils and plant varieties [9, 10] and when analysing the rhizosphere of plants of different age [9].

Cluster analysis and principal component analysis (PCoA). In the dendrogram (Fig. 1), «chernozem cluster» (including the initial chernozem and the rhizosphere of both plants formed on this soil) and "sod-podzolic cluster" clearly stand out. The first one demonstrated a pronounced division of communities into two separate groups which corresponded to the initial soil and rhizosphere. In this case, communities of rye and wheat rhizospheres in chernozem in terms of their taxonomic structure were more similar to each other than each of them to the initial soil community. In the «sod-podzolic cluster» another trend is observed, i.e. the initial soil and rhizosphere of rye on sod-podzolic soil appeared to be in a separate clade. These data correlate well with diversity indices, further suggesting an expressed rhizosphere effect in the cultivation of wheat. However, the observed effect, associated with a reduced diversity in the rhizosphere of wheat on the sod-podzolic soil, can be considered only as a trend, since the corresponding cluster on the dendrogram had a relatively low statistical support (no more than 64 %). However, it was reproduced in the principal component analysis.

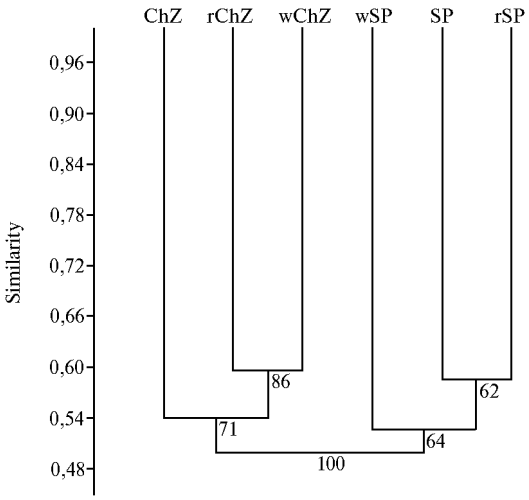

Fig. 1. Cluster analysis of microbiomes of soils and rhizosphere communities formed in these soils: ChZ and SP — chernozem and sod-podzolic soil; rChZ and rSP — rhizosphere of rye (*Secale cereale* L., a local variety from Pskov Province, k-6469 in the VIR catalog, N.I. Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg;), wChZ and wSP — rhizosphere of wheat (*Triticum aesivum* L., the Volshebnitsa variety). Greenhouse pot experiments. The similarity dendrogram was constructed using the Bray-Curtiss similarity measure, bootstrap = 1,000.

The graph, representing the results of the PCoA, clearly shows marked differences in the structure of clusters corresponding to the chernozem and sod-podzolic soils, i.e.

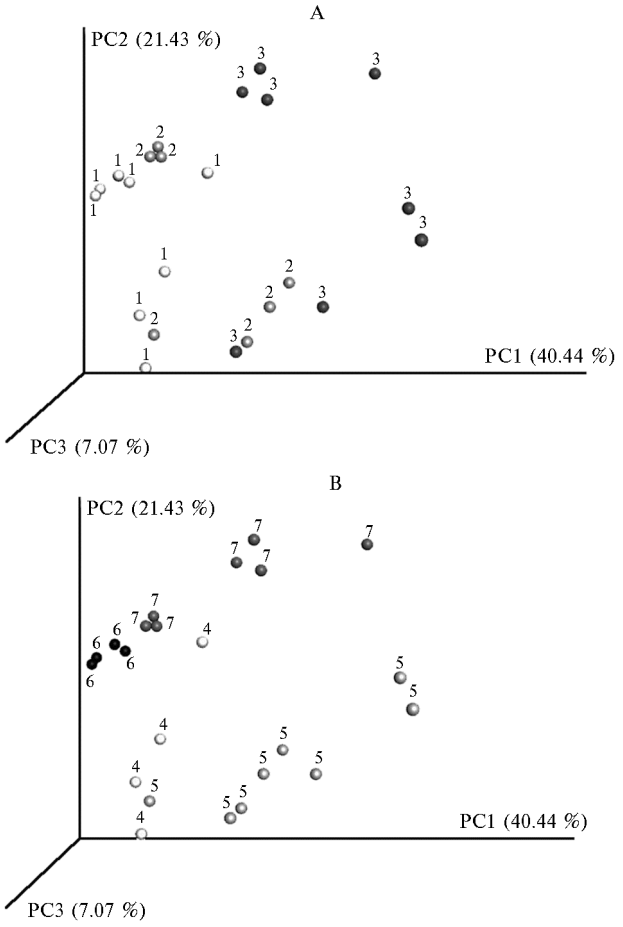

Fig. 2. Principal component analysis (PCoA) **of soil communities and based on them rhizosphere microbial communities:** A — compared to the initial soil, B — in initial soils and rhizospheres based on them; 1 — initial soil, 2 — rhizosphere of rye (*Secale cereale* L., a local variety from Pskov Province, k-6469 in the VIR catalog,) 3 — rhizosphere of wheat (*Triticum aesivum* L., the Volshebnitsa variety); 4 — chernozem, 5 — rhizospheres on chernozem, 6 — sod-podzolic soil, 7 — rhizospheres on sod-podzolic soil. Greenhouse pot experiments.

in the first case, we observed a significant variation in experiment replications,

while in the second one the variation was practically absent (Fig. 2). The reason may lie in the high heterogeneity of the soil. Meanwhile, it is possible that the sequencing depth was inadequate for the community. Also, the graph shows that communities of the rye rhizospheres on the sod-podzolic soil were similar in structure to the communities of the initial soil, and the differences were seen only in communities of the wheat rhizospheres which constituted a separate group (see Fig. 2, A). There were no clearly separate groups determined in the chernozem communities. However, as we can see, the communities of rye rhizospheres have been generally closer to the communities of the initial soils than the communities of wheat rhizospheres.

The taxonomic composition of communities. An analysis, carried out at the phyla-level (classes for the phylum *Proteobacteria*), showed significant differences ($p < 0.05$) in the number of sequences between the experiment variants (Fig. 3).

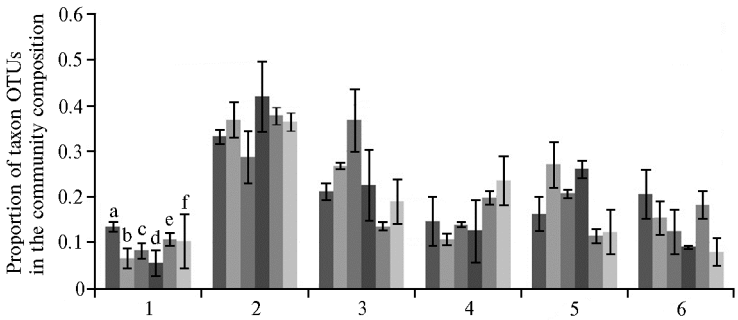

Fig. 3. Taxonomic composition (presented in part) **of soil communities and based on them rhizosphere microbial communities:** OTU — operational taxonomic unit; 1 — *Acidobacteria*, 2 — *Actinobacteria*, 3 — *Bacteroidetes*, 4 — *Verrucomicrobia*, 5 — *Betaproteobacteria*, 6 — *Gammaproteobacteria*; a — rye (*Secale cereale* L., a local variety from Pskov Province, k-6469 in the VIR catalog,), sod-podzolic soil; b — rye, chernozem; c — wheat (*Triticum aesivum* L., the Volshebnitsa variety), sod-podzolic soil; d — wheat, chernozem; e — control, sod-podzolic soil; f — control, chernozem. Greenhouse pot experiments, $p < 0.05$.

All rhizosphere communities showed an increase in the number of sequences belonging to a class of *Betaproteobacteria* (non-significant for SP and rSP communities), as well as a reduced number of representatives of the *Verrucomicrobia* phylum (non-significant for rSP—SP and wChZ—ChZ communities). A significantly increased number of sequences belonging to a class *Gammaproteobacteria* was evident for the sod-podzolic soil community as compared to the chernozem community, although this trend was lost in the communities of the rhizospheres of these soil types. In the communities of the wheat rhizosphere on the sod-podzolic soil, it was observed an increase in the proportion of representatives of the *Bacteroidetes* phylum. The proportion turned out to be significantly lower in the community of the rye rhizosphere in the sod-podzolic soil, and even lesser in the very sod-podzolic soil. It has also been shown a decrease in the number of representatives of the *Actinobacteria* phylum in the wheat rhizosphere on the sod-podzolic soil compared to the initial soil. The communities of the rye rhizosphere on the sod-podzolic soil differed from wheat rhizosphere communities on the same soil with a significantly higher number of representatives of the *Actinobacteria* phylum.

As it is known from the literature data, at different developmental stages, plants possess mostly different groups of microorganisms. Based on the study of root exudation in *Arabidopsis*, the major differences were found in four phyla, such as *Acidobacteria*, *Actinobacteria*, *Bacteroidetes* and *Cyanobacteria*, moreover, a positive correlation was revealed between the number of the *Bacteroidetes* phy-

lum representatives with the number of amino acids released by the roots of this plant, and an inverse correlation with the phenolic compounds [11].

Most likely, it is an increase in the number of the *Bacteroidetes* phylum representatives in the wheat rhizosphere community on the sod-podzolic soil, which led to a marked difference in terms of α-diversity in comparison to the initial soil. The statistical analysis revealed that differences in the composition of communities in this case were associated with an increase (more than 12.6-fold, $p < 0.05$) in the number of microorganisms from the *Flavobacterium* genus.

A more detailed statistical analysis of the taxonomic structure of microbial communities also allowed to identify the group of microorganisms which caused differences in the formation of rhizosphere microbiomes in other variants of the experiment. In particular, compared to the initial soil (ChZ), in the rChZ rhizosphere, there was a significant increase in the proportion of the *Pedobacter* (by 47.6 times) and *Chitinophaga* (by 76.9 times) genera, and in the wChZ rhizosphere — of the *Pedobacter* (10.0-fold) and *Kaistobacter* (14.2-fold) genera. In the rSP rhizosphere (as compared to SP), there was an increase in the proportion of the *Pseudomonas* (by 48.6 times) and *Achromobacter* (by 25.0 times) genera, and in the wSP rhizosphere — of the *Mesorhizobium* (40.0 times) and *Chitinophaga* (53.7 times) genera. Therefore, the composition of the rhizosphere community varied in different soils. These results are consistent with the literature data indicating that the rhizospheres of plants cultivated on soils of different types vary significantly in composition at the genera-level. For example, growing lettuce on three different soils showed an increase in the number of members of the *Sphingomonas* and *Rhizobium* (α-proteobacteria), *Pseudomonas* (γ-proteobacteria), *Variovorax* (β-proteobacteria) and *Flavobacterium* (*Bacteroidetes*) families, however, unique families are typical for each of the soil type which alter the number of their representatives only in rhizospheres on this type of soil [11].

It should be emphasized that currently there is no single internationally recognized method both for separating communities of the rhizosphere and rhizoplane, and for the isolation of DNA from the soil and its further analysis. Therefore, our findings are difficult to be compared with those described in the literature. Note, for example, that a more thorough analysis of the communities of the root zone, separating the rhizosphere and the rhizoplane, indicates that the rhizosphere community has a similar taxonomic composition with the community of the initial soil, while the major differences are observed in the rhizoplane, which are limited to a decrease in the proportions of the *Acidobacteria*, *Planctomycetes*, and *Gemmatimonadetes* phyla [24]. It is now clear that, for a more comprehensive assessment of the rhizosphere effect, a universal sampling technique should be developed which would include additional methods enabling a more precise localization of some representatives of the rhizosphere community (e.g., FISH techniques — fluorescence in situ hybridization, etc.).

Therefore, despite the relatively short duration of the experiment (42 days), the communities of the initial soils and rhizospheres varied greatly, i.e. a pronounced rhizosphere effect was revealed in both types of soil. The formation of the rhizosphere community is greatly influenced by both a soil type and the plant species. The strongest factor appeared to be the type of soil, since rhizosphere communities, generated in various types of soil, and the initial soil communities significantly differ from each other. As it was demonstrated, these differences were preserved for both soils during the formation of different rhizosphere communities. The plant species was the second most important (after the type of soil) factor in determining the taxonomic composition of the rhizosphere microbiome. In general, the communities of the rye rhizosphere are somewhat closer to the communities of the initial soils than the wheat rhizosphere communities are.

The taxonomic analysis of the communities at the phyla-level allowed to reveal groups, most responsible for the rhizosphere effect, i.e. the formation of rhizosphere communities was accompanied by an increase in the number of sequences from the *Betaproteobacteria* class along with reductions in the number of the *Verrucomicrobia* phylum representatives.

The combination of features of the sod-podzolic soil and characteristics of the wheat, cultivated on it, resulted in significant changes in the community. According to the results of all analyzes, these communities differ significantly from the initial soil communities and the communities of the rye rhizosphere on the sod-podzolic soil. This may be due to an increase in the proportion of bacteria from the *Flavobacterium* genus (the *Bacteroidetes* phylum) in these communities.

Thus, using high-throughput sequencing method with its high resolution and the ability to examine even non-culturable microorganisms, the rhizosphere effect was shown to exist in the soil when growing cultivars. However, to confirm the presence of this effect, as well as for more detailed studies of mechanisms underlying it, our taxonomic analysis should be supported by further studies that would characterize the association between the structure of the rhizosphere microbiome and the composition of plant exudates. To do this, model experiments are projected on introducing into the soil the components of plant exudates of the tested rye and wheat varieties.

REFERENCES

1. Kostychev S.P. *Fiziologiya rastenii. Chast'. 2* [Plant physiology. Part 2]. Moscow-Leningrad, 1933 (in Russ.).
2. Genkel' P.A. *Fiziologiya rastenii s osnovami mikrobiologii* [Plant physiology and fundamentals of microbiology]. Moscow, 1962 (in Russ.).
3. Philippot L., Raaijmakers J.M., Lemanceau P., van der Putten W.H. Going back to the roots: the microbial ecology of the rhizosphere. *Nat. Rev. Microbiol.*, 2013, 11(11): 789-799 (doi: 10.1038/nrmicro3109).
4. Rovira A.D. Plant root exudates. *Bot. Rev.*, 1969, 35: 35-57.
5. Hale M.G., Moore L.D., Griffin G.J. Root exudates and exudation. In: *Interaction between non-pathogenic soil microorganisms and plants.* Y.R. Dommergues, S.V. Krupa (eds.). Amsterdam, 1978: 163-203.
6. Tikhonovich I.A., Provorov N.A. *Simbiozy rastenii i mikroorganizmov: molekulyarnaya genetika agrosistem budushchego* [Microbial-plant symbiosis: molecular genetics of prospective agrosystems]. St. Petersburg, 2009 (in Russ.).
7. Kravchenko L.V., Shaposhnikov A.I., Makarova N.M., Azarova T.S., L'vova K.A., Kostyuk I.I., Tikhonovich I.A. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2011, 3: 71-75. Available http://www.agrobiology.ru/3-2011kravchenko.html. No date (in Russ.).
8. Shaposhnikov A.I., Belimov A.A., Kravchenko L.V., Vivanko D.M. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2011, 3: 16-22. Available http://www.agrobiology.ru/3-2011shaposhnikov.html. No date (in Russ.).
9. Bais H.P., Weir T.L., Perry L.G., Gilroy S., Vivanco J.M. The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu. Rev. Plant Biol.*, 2006, 57: 233-266 (doi: 10.1146/annurev.arplant.57.032905.105159).
10. Wieland G., Neumann R., Backhaus H. Variation of microbial communities in soil, rhizosphere, and rhizoplane in response to crop species, soil type, and crop development. *Appl. Environ. Microbiol.*, 2001, 67(12): 5849-5854 (doi: 10.1128/AEM.67.12.5849-5854.2001).
11. Schreiter S., Ding G.C., Heuer H., Neumann G., Sandmann M., Grosch R., Kropf S., Smalla K. Effect of the soil type on the microbiome in the rhizosphere of field-grown lettuce. *Front. Microbiol.*, 2014, 5: 144 (doi: 10.3389/fmicb.2014.00144).
12. Chaparro J.M., Badri D.V., Vivanco J.M. Rhizosphere microbiome assemblage is affected by plant development. *ISME J.*, 2014, 8(4): 790-803 (doi: 10.1038/ismej.2013.196).
13. Winston M.E., Hampton-Marcell J., Zarraonaindia I., Owens S.M., Moreau C.S., Gilbert J.A., Hartsel J.A., Kennedy S.J., Gibbons S.M. Understanding cultivar-specificity and soil determinants of the *Sannabis* microbiome. *PLoS ONE*, 2014, 9(6): e99641 (doi: 10.1371/journal.pone.0099641).
14. Bulgarelli D., Garrido-Oter R., Münch P.C., Weiman A., Dröge J., Pan Y., McHardy A.C., Schulze-Lefert P. Structure and function of the bacterial root microbiota in wild and domesticated barley. *Cell Host Microbe*, 2015, 17(3): 392-403 (doi: 10.1016/j.chom.2015.01.011).

15. Coleman-Derr D., Desgarennes D., Fonseca-Garcia C., Gross S., Clingenpeel S., Woyke T., North G., Visel A., Partida-Martinez L.P., Tringe S.G. Plant compartment and biogeography affect microbiome composition in cultivated and native *Agave* species. *New Phytol.*, 2016, 209(2): 798-811 (doi: 10.1111/nph.13697).
16. Handelsman J. Metagenomics: application of genomics to uncultured microorganisms. *Microbiol. Mol. Biol. Rev.*, 2004, 68: 669-685 (doi: 10.1128/MMBR.68.4.669-685.2004).
17. Lundberg D.S., Lebeis S.L., Paredes S.H., Yourstone S., Gehring J., Malfatti S., Tremblay J., Engelbrektson A., Kunin V., delRio T.G., Edgar R.C., Eickhorst T., Ley R.E., Hugenholtz P., Tringe S.G., Dangl J.L. Defining the core *Arabidopsis thaliana* root microbiome. *Nature*, 2012, 488(7409): 86-90 (doi: 10.1038/nature11237).
18. Micallef S.A., Shiaris M.P., Colyn-Carmona A. Influence of *Arabidopsis thaliana* accessions on rhizobacterial communities and natural variation in root exudates. *J. Exp. Bot.*, 2009: 60(6): 1729-1742 (doi: 10.1093/jxb/erp053).
19. van der Heijden M.G., Schlaeppi K. Root surface as a frontier for plant microbiome research. *PNAS USA*, 2015, 112(8): 2299-2300 (doi: 10.1073/pnas.1500709112).
20. Bonito G., Reynolds H., Robeson M.S. 2nd, Nelson J., Hodkinson B.P., Tuskan G., Schadt C.W., Vilgalys R. Plant host and soil origin influence fungal and bacterial assemblages in the roots of woody plants. *Mol. Ecol.*, 2014, 23(13): 3356-3370 (doi: 10.1111/mec.12821).
21. Bodenhausen N., Horton M.W., Bergelson J. Bacterial communities associated with the leaves and the roots of *Arabidopsis thaliana*. *PLoS ONE*, 2013, 8(2): e56329 (doi: 10.1371/journal.pone.0056329).
22. Neumann G., Bott S., Ohler M.A., Mock H.P., Lippmann R., Grosch R., Smalla K. Root exudation and root development of lettuce (*Lactucasativa* L. cv. Tizian) as affected by different soils. *Front. Microbiol.*, 2014, 5: 2 (doi: 10.3389/fmicb.2014.00002).
23. Donn S., Kirkegaard J.A., Perera G., Richardson A.E., Watt M. Evolution of bacterial communities in the wheat crop rhizosphere. *Environ. Microbiol.*, 2015, 3: 610-621 (doi: 10.1111/1462-2920.12452).
24. Edwards J., Johnson C., Santos-Medellín C., Lurie E., Podishetty N.K., Bhatnagar S., Eisen J.A., Sundaresan V. Structure, variation, and assembly of the root-associated microbiomes of rice. *PNAS USA*, 2015, 112(8): E911-E920 (doi: 10.1073/pnas.1414592112).
25. Andronov E.E., Pinaev A.G., Pershina E.V., Chizhevskaya E.P. *Nauchno-metodicheskie rekomendatsii po vydeleniyu vysokoochishchennykh preparatov DNK iz ob"ektov okruzhayushchei sredy* [Isolation of highly purified DNA from environmental objects: guidelines]. St. Petersburg, 2011 (in Russ.).
26. Bates S.T., Berg-Lyons D., Caporaso J.G., Walters W.A., Knight R., Fierer N. Examining the global distribution of dominant archaeal populations in soil. *ISME J.*, 2011, 5: 908-917 (doi: 10.1038/ismej.2010.171).
27. Caporaso J.G., Kuczynski J., Stombaugh J., Bittinger K., Bushman F.D., Costello E.K., Fierer N., Peca A.G., Goodrich J.K., Gordon J.I., Huttley G.A., Kelley S.T., Knights D., Koenig J.E., Ley R.E., Lozupone C.A., McDonald D., Muegge B.D., Pirrung M., Reeder J., Sevinsky J.R., Turnbaugh P.J., Walters W.A., Widmann J., Yatsunenko T., Zaneveld J., Knight R. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, 2010, 7(5): 335-336 (doi: 10.1038/nmeth.f.303).
28. DeSantis T.Z., Hugenholtz P., Larsen N., Rojas M., Brodie E.L., Keller K., Huber T., Dalevi D., Hu P., Andersen G.L. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl. Environ. Microbiol.*, 2006, 72(7): 5069-5072 (doi: 10.1128/AEM.03006-05).
29. Lozupone C.A., Knight R. Global patterns in bacterial diversity. *PNAS USA*, 2007, 104(27): 11436-11440 (doi: 10.1073/pnas.0611525104).
30. Hammer O., Harper D., Ryan P. PAST: Paleontological Statistics software package for education and data analysis. *Paleontologia Electronica*, 2001, 4(1): art. 4 (9pp).