Yu.P. BORKO[1]*, **M.V. PATYKA**[2], **M.V. BOIKO**[2], **A.M. HONCHAR**[2], **V.M. SINCHENKO**[3]

[1] NSC "Institute of Agriculture of NAAS of Ukraine",
2-B Machynobudivnykiv Str., Chabany, Fastiv Raion, Kyiv Oblast 08162, Ukraine
[2] National University of Life and Environmental Sciences of Ukraine,
13 Heroiv Oborony Str., Kyiv, 03041, Ukraine
[3] Institute of Bioenergetic Cultures and Sugar Beets of NAAS of Ukraine,
25 Clinichna Str., Kyiv, 03141, Ukraine
* Author for correspondence; e-mail: yulia_borko@ukr.net

THE FEATURES OF TAXONOMIC STRUCTURE FORMATION OF SOIL MICROBIAL BIOME IN *BETA VULGARIS* RHIZOSPHERE

The necessity to increase the production of high-quality agricultural products in order to minimize using of agrochemicals while maintaining high profitability of production requires a comprehensive study of the determining factor of soil fertility, its biological component. Research of the microbiocenoses formation in the plants' rhizosphere at all ontogenesis stages will allow to uncover the mechanisms of microbial-plant interaction and develop effective ways to increase crop productivity with high functional activity and homeostasis of the soil microbiome. **The aim of the work** *is to investigate the structure of the microbial complex and biodiversity of Beta vulgaris rhizosphere during ontogenesis by classical microbiological and molecular-biological methods.* **Methods.** *The number of microorganisms was determined by the method of inoculation soil microbial suspension on agar nutrient media. The structure of the qualitative composition of microorganisms was identified by morphologically-cultural properties and the morphology of isolated isolates — by microscopy of fixed preparations. The diversity of soil microbial complexes was evaluated by the Shannon, Simpson, and Berger-Parker ecological indices. The taxonomic structure of prokaryotes was determined by pyrosequencing.* **Results.** *The differentiation of the soil microbiota number was observed during the Beta vulgaris ontogenesis due to the intensive production of root exudates by the plant. The number of bacteria and micromycetes increased by 1.8—2.3 times, however, in the phase of leaves closing in-row spacing, the number of fungal microbiota decreased by 46.4%. Microbial complexes differed in the number of detected morphotypes (27—50) and in the structure of the*

*distribution of dominant forms (the total number of dominant forms of bacteria decreased during the growing season, whereas that of micromycetes increased). Analysis of the prokaryotes metagenome by pyrosequencing made it possible to identify 214 operational taxonomic units, 10.1% of which are forms that are not cultivated on nutrient media, and 23.3% are unclassified. Among the identified taxonomic units, 96.2% were identified at the order level, 85.7% — at the family level, and 76.7% — at the genus level. Among the identified taxonomic units were 15 phyla bacteria and 1 archaea, among which 96 taxonomic units, families — 167, genera — 214 we found at the level of microbial orders. The dominant phyla forms were Proteobacteria (65.7%) and Actinobacteria (20.5%); orders — Burkholderiales (38.7%) and Pseudomonadales (20.1%); families — Alcaligenacea (37.9%), Pseudomonadaceae (20.1%); Gaiellaceae (5.7%), Nitrososphaeraceae (4.2%); generas — Achromobacter (31.5%) and Pseudomonas (19.9%). According to the indicators of ecological indices determined on the basis of the results of classical microbiological and molecular biological research methods, it was established that the microbial complex of the soil is characterized by high biodiversity, although the Shannon (I_{Sh} = 5.36) and Simpson (I_S = 0.87) indices based on the pyrosequencing method results were significantly higher than similar indicators identified by classical microbiological methods. **Conclusions**. During the ontogenesis of Beta vulgaris, including due to the intensive production of root exudates by the plants, the number of bacteria and micromycetes in the rhizosphere of plants increases. It is accompanied by a redistribution of structural composition and an increase in the microorganisms' diversity (I_{Sh} = 5.36). It has been found that among the identified 214 taxonomic units, 10.1% are forms that are not cultivated on nutrient media, and 23.3% are unclassified. Our studies show that the structure of the microbial complex of the plants' rhizosphere reflects the characteristics of the soil and can be used as an indicator of ecological status. The work results (obtained for the first time in the Forest-Steppe of Ukraine) deepen the knowledge of the true scale of natural genetic diversity of microbial complexes and are a valuable asset for substantiating practical proposals for effective adaptive interactions in the plant-microorganism system to preserve the homeostasis of agroecosystems.*

Keywords: metagenome, taxonomic structure, pyrosequencing, morphotypes, rhizosphere, sugar beet, ontogenesis, biodiversity indices.

The most urgent task of agricultural production today is the increasing production of safe and high-quality agricultural products against the background of minimizing the use of mineral fertilizers and chemical plant protection products [1]. Since the determining component of crop formation is a biological component of soil (microbiocenosis) and interaction in the system "soil — microorganisms — plant" includes the functioning and agroecosystems' homeostasis, it is obvious that the study of microbiome formation in the plant rhizosphere will allow revealing the mechanisms of loading the soil microbiome [2]. The information on changes in the structure of microorganisms is important in this context throughout the plants' ontogenesis and the impact on them of the plant itself, that is, the root exudates [3]. It will reveal the mechanisms of plant-microbial interaction and develop effective systems to increase the productivity of the crops against the background of high functional activity of the soil microbial component [4].

Microbial biome and soil metagenome are complex systems of interacting organisms, extremely diverse and numerous in species number, functional activity, and ecological role in the environment [5]. Classical microbiological methods based on the cultivation of microorganisms are important for studying the ecology of soil microbiota, their functional activity in natural ecosystems, and agrophytocenoses [6]. They are the primary link in the assessment of the soil microbial complex but are not very informative for assessing microbial genetic diversity by selecting a specific population of microorganisms, as they allow assessing only 0.1—10.0% of the soil microbial stock [7]. The use of molecular-biological methods for the assessment of soil microorganisms allows one to study the microbial cenosis at the phylogenetic level, as well as to identify species of soil microbiota that are unknown to science and are not cultivated on ordinary nutrient media [8]. However, studies of the soil microbial biome and metagenome structure using molec-

ular-biological methods are mostly fragmentary because today the methodological support is insufficient. There is no comprehensive description of vegetation, soil and climatic conditions, as well as applied agricultural measures that determine the functioning and formation of microbial groups, their structure, and biodiversity during plant ontogenesis [9]. In this regard, it is difficult to conduct any comparative analysis of data on taxonomic structure and microbial complexes diversity in agroecosystems obtained by different researchers. Therefore, a complex combination of classical and molecular genetic methods of analysis is necessary to study the structure and biodiversity of soil microbiome formed in the crops' ontogenesis. The obtained results can be used to assess and optimize new environmentally reasonable agricultural measures focused on increasing and disclosing the crops' productive potential under conditions of high-profitable production and maintaining soil fertility.

The **aim** of the work is to investigate the structure of the microbial complex and biodiversity of *Beta vulgaris* rhizosphere during culture ontogenesis by classical microbiological and molecular-biological methods.

Materials and methods. Studies of the microbial complex of *Beta vulgaris* (sugar beet) rhizosphere were conducted based on the National University of Life and Environmental Sciences of Ukraine (NULES) "Agronomic Research Station" in the Fastiv raion in the Kyiv oblast. Soil cancellation is represented by chornozem typical low-humus coarse-dusty-loamy. Soil samples were prepared in the main phases of culture ontogenesis: germination, leaves closing in-row spacing, and technical maturity.

The number of microorganisms was determined by the method of seeding of soil suspensions on agar nutrient media and expressed by the number of Colony-Forming Units (CFU) in 1 g of dry soil [10]. The structure of the qualitative composition of soil microorganisms was studied by conventional methods for morphologically-

cultural properties. Basing on the mathematical calculations of the frequency of morphotypes, we identified the following groups of microorganisms: dominant (> 10%), subdominant (5—10%), frequent (1—5%), and rare (<1%) [11]. The diversity of soil microbial complexes was evaluated using ecological indices of Shannon, Simpson, and Berger-Parker [12]. The prokaryotes taxonomic structure of typical chernozem was determined by the pyrosequencing method, which includes the following steps: creating a library with fluorescent primers; double purification of the PCR product; emulsion PCR; pyrosequencing; nucleotide sequence analysis; identification of the taxonomic structure of microbiological complexes and their comparative analysis [13, 14]. Sample preparation, emulsion PCR, and sequencing were performed on a GS Junior instrument (Roche, USA) according to the guidelines of manufacturers [15, 16]. Computer processing of nucleotide sequences obtained by sequencing was carried out on the software module QIIME version 1.7.0 [17]. Statistical processing and mathematical analysis of research results were realized in MS Excel 10.0 and STATISTICA 7.0.

Results. The quantitative composition of microorganisms is an integral feature of a comprehensive analysis of rhizosphere microbiota [1]. Microbiological studies of the plants' rhizosphere on the example of *Beta vulgaris* have shown that the number of bacterial and fungal microbiota is related to the peculiarities of plant growth and development. Thus, the number of bacteria and micromycetes in the rhizosphere was the lowest (9.06 million and 30.35 thousand CFU/g of soil, respectively) in the germination phase (Table 1). This It is due to the low production intensity of plant root exudates. The number of root secretions increased with the plant growth and development (phase of leaves closing in-row spacing), which was accompanied by an increase in the bacteria number by 77.5% compared to their number at the beginning of the growing season. However, the number of mi-

cromycetes decreased by 46.4%, which led to the reduction in the plant residues with high fiber content in the topsoil. At the end of the growing season (technical maturity) in the rhizosphere fresh dead organic residues were accumulated, which led to a further increase in the bacteria number (18.86 million CFU) along with a significant increase in micromycetes (76.03 thousand CFU) and contributed to the intensification of soil organic compounds.

Analysis of the microbiota qualitative composition of *Beta vulgaris* rhizosphere based on the description of morphologically-cultural properties of microorganisms in the main phases of culture ontogenesis showed that the studied microbial complexes differ in the number of identified morphotypes and the structure of distribution of dominant forms of microorganisms (Figs. 1—3).

Thus, in the germination phase, 36 morphotypes of bacterial and 27 — fungal microbiota with a saturation of 1.59—23.44% were evaluated (Fig. 1). Regarding the structure of the distribution of detected morphotypes, it was found that the largest share of bacteria (86.1%) and micromycetes (77.8%) were morphotypes, which are "common", the share of dominants was 5.6 and 7.4%, subdominants — 8.3 and 14.8% in accordance (Fig. 2).

During the leaves' closing phase, the structure of the microbial complex was redistributed between rows, which is due to the intensive *exudate production by plants.* Thus, the total number of bacterial morphotypes increased by 25.0**%**, and the diversity of micromycete morphotypes

decreased by 58.8% (Figs. 1, 2). Besides, in the middle of the growing season, random species of microbiota were found in the structure of the microbial complex of the Beta vulgaris rhizosphere, which were classified as "others". They are not permanent representatives of the microbial complex and are activated only in the presence of a significant amount of easily digestible nutrients in the soil (exudates, plant remains). Their saturation among the total number of bacterial morphotypes was 37.8%, and that for micromycetes was 27.5%. Morphotypes that "often occur" occupied the largest share of microbiota (bacteria 48.9%, micromycetes 41.2%), like in the germination phase.

In the technical maturity phase of *Beta vulgaris,* the qualitative diversity of bacterial and fungal microbiota continued to increase by 11.1 and 64.7%, respectively, compared to the leaves closing in-row spacing phase. This is due to the accumulation of organic matter in the soil through underground and aboveground plant biome. Thus, the number of bacterial morphotypes was 50 CFU, micromycetes — 28 CFU (Fig. 1). At the same time, the *diversity increase for bacterial* and micromycete morphotypes at the end of the *Beta vulgaris* vegetation was due to the increase in the number of "frequent" representatives (up to 53.6%) and "others" (up to 37.8%), which was associated with the increasing amount of readily available organic compounds that contribute to the microbial diversity formation (Fig. 2).

The distribution of dominant representatives of microbiota in typical chornozem in *Beta vulgaris* ontogenesis was not uniform: the total number of bacteria dominant forms decreased, while that of micromycetes increased during the culture vegetation (Fig. 3). This is due to the culture peculiarities and indicates the homeostatic microbial coenoses formation in the soil. At the beginning of plant ontogenesis, dominant forms of bacteria and micromycetes were identified as respectively 2 and 1 during the period of active culture development and 1 and 4 at the end of the growing season (Fig. 3). This testifies

Table 1. **The number of bacteria and micromycetes in *Beta vulgaris* rhizosphere**

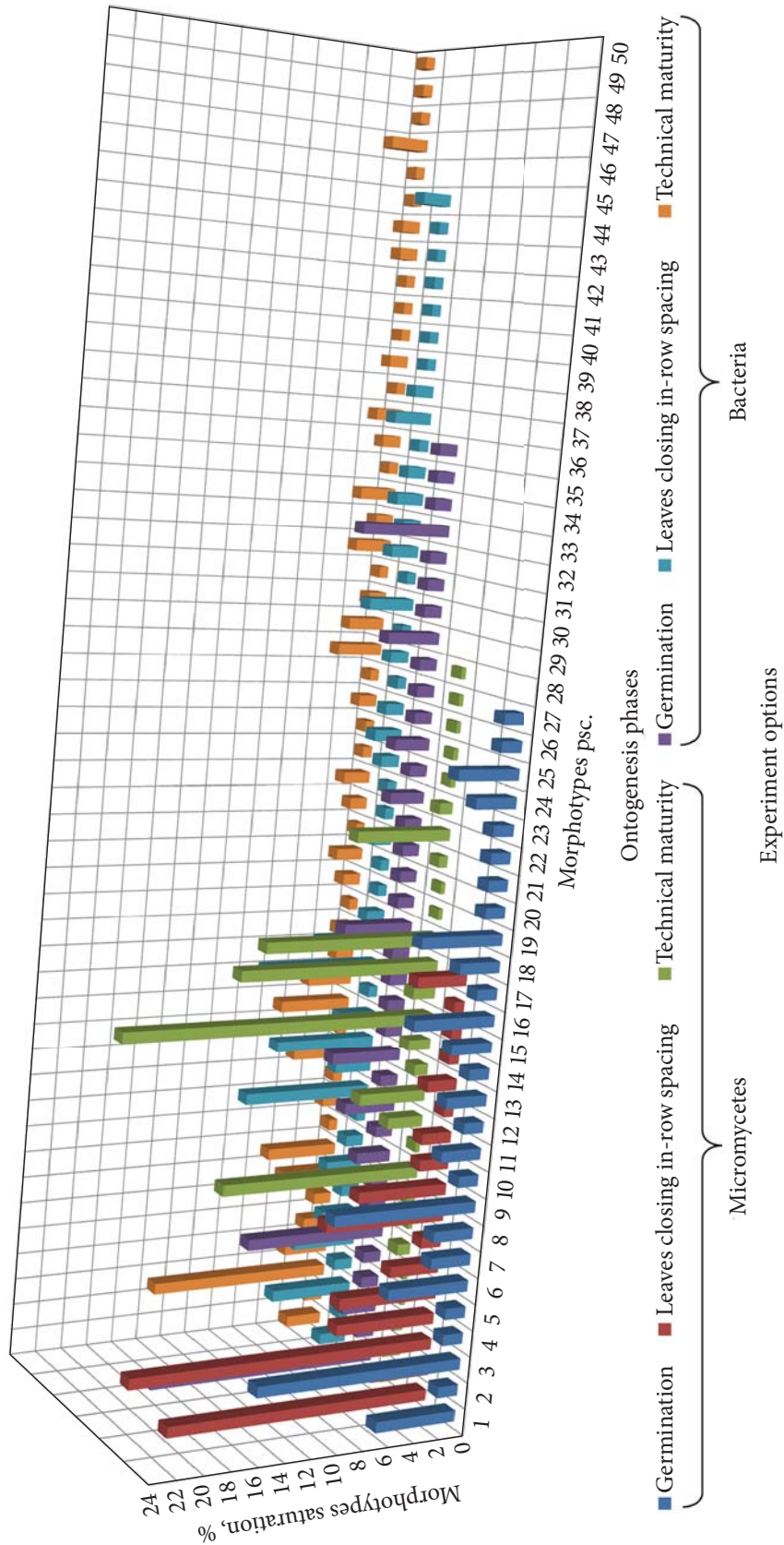| Phases of plant ontogenesis	Bacteria, mln CFU/g of soil	Micromycetes, ths CFU/g of soil
Germination	9.06±0.75	30.35±1.56
Leaves closing in-row spacing	16.08±1.03	20.73±1.29
Technical maturity	18.86±1.00	76.03±3.76

Fig. 1. The structure of micromycetes and bacteria morphotypes in the *Beta vulgaris* rhizosphere
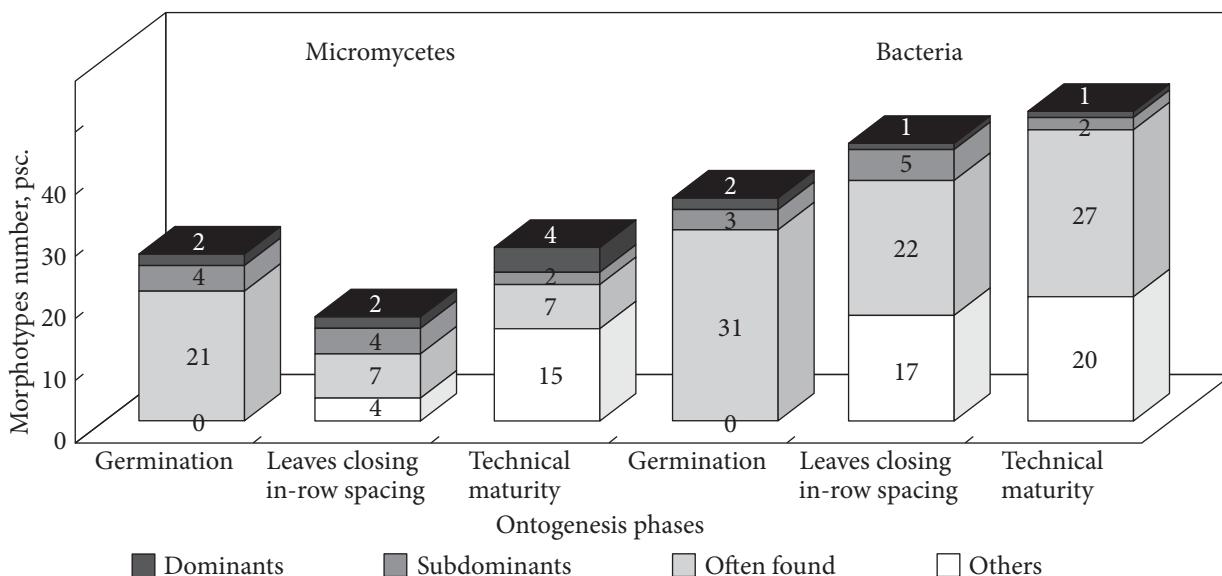
Fig. 2. Distribution of detected morphotypes of bacterial and fungal microbiota in the *Beta vulgaris* rhizosphere
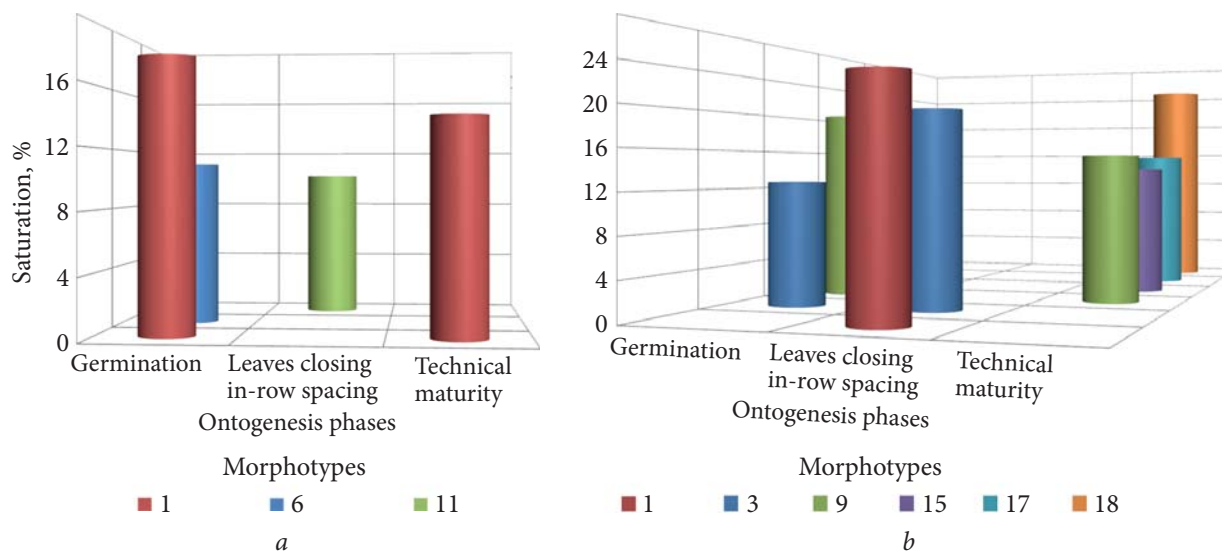

Fig. 3. Distribution of dominant morphotypes of bacteria (*a*) and micromycetes (*b*) of in the *Beta vulgaris* rhizosphere

to the formation of a homogeneous soil microbial complex with a high degree of micromycete dominance.

To assess the species diversity of microbiota, the following environmental indicators were determined: the indices of species richness of Shannon, Simpson, and the index of the Berger-Parker dominance. Indicators of the Shannon index, which determines the degree of uniformity in of the distribution of traits of the sample objects, have shown a high diversity of bacteria (I_{Sh} = 1.36-1.43) and 7.9—38.8% lower diversity of micromycetes (I_S = 1.02—1.26) (Table 2). During the growing season, the diversity of bacterial microbiota increased slightly, while that of fungal microbiota decreased.

Indicators of the Simpson index, which point to the share in the species composition of the biocenosis occupied by common, "background" species, range from 0.05 to 0.06 (bacteria) and from 0.06 to 0.13 (micromycetes, germination phase) and indicate a uniform distribution of representatives of the microbial complex (Table 2). This index increased for micromycetes (phases of leaves closing in-row spacing and technical maturity) to 0.12 and 0.13 respectively, which indicates a decrease in the species richness of the fungal microbiota and is consistent with previously obtained data (Shannon index).

The Berger-Parker index, which characterizes the relative importance of the most numerous species, was 0.10—0.17 for bacteria and 0.16—0.24 for micromycetes. This indicates a more uniform distribution of the bacterial population compared to the fungal one (Table 2). The increasing Berger-Parker index for micromycetes during culture ontogenesis indicates a microbiota diversity decrease due to the increasing dominance degree of some morphotypes. However, due to the increase in the number of representa-

Table 2. **Ecological indexes of diversity and dominance of the microbiota in *Beta vulgaris* rhizosphere**

Phases of plant ontogenesis	Indices		
	Shannon	Simpson	Berger-Parker
Bacteria			
Germination	1.36	0.06	0.14
Leaves closing in-row spacing	1.39	0.06	0.10
Technical maturity	1.43	0.05	0.17
Micromycetes			
Germination	1.26	0.06	0.16
Leaves closing in-row spacing	1.02	0.12	0.23
Technical maturity	1.03	0.13	0.24

tive active forms of random soil microbiome species, the diversity of micromycetes was preserved.

There is observed an inverse correlation (r = = –0.90 for bacteria, r = –0.99 for micromycetes) between the Simpson and Shannon indices during the growing season. That indicates the formed systems of microbial complexes and confirms the reliability of the obtained data.

The diversity of prokaryotes metagenome of typical chernozem was detected and evaluated by a pyrosequencing method for the first time in the Forest-Steppe of Ukraine to assess the taxonomic structure of the microbiome of the rhizosphere of sugar beet. The analysis of prokaryotes metagenome by pyrosequencing made it possible to identify 214 operational taxonomic units, 10.1% of which are forms that are not cultivated on nutrient media, 23.3% are unclassified, but they are functionally significant in the rhizosphere. Among the identified taxonomic units, 96.2% were identified at the order level, 85.7% — at the family level, and 76.7% — at the genus level. Among the identified taxa, 15 phyla were bacteria, and 1 was archaea. To the dominant taxa, we included representatives of the microbiota, the share of which was > 10%; to the subdominant taxa — 1—10%. The dominant forms among the identified phyla were representatives of the bacteria *Proteobacteria* (65.7%) and *Actinobacteria* (20.5%); subdominant — *Chloroflexi* (2.3%), *Acidobacteria* (1.9%), *Gemmatimonadetes* (1.2%), and archaea *Crenarchaeota* (4.2%). The share of unclassified sequences was 1.0% of their total number (Fig. 4).

The study of the microbiome at the microbial orders level, which was detected 96, showed the absolute dominance of *Burkholderiales* (subgroup *β-Proteobacteria*) with a content of 38.7% of the total number of detected prokaryotes and *Pseudomonadales* (*γ-Proteobacteria)* with a content of 20.1% (Fig. 5).

The subdominant orders included *Solirubrobacterales* (6.5%), *Gaiellales* (5.8%), *Actinomycetales* (4.7%), *Nitrososphaerales* (4.2%). The

Fig. 4. Distribution of the main phyla of the soil microbial complex

Crenarchaeota — Archaea
Acidobacteria
Actinobacteria
Chloroflexi
Firmicutes
Gemmatimonad
Planctomycetes
Proteobacteria
Verrucomicrobia
Not known to science
Not cultivated
} Bacteria

Fig. 5. The metagenome of the prokaryotic complex of soil at the orders level

Nitrososphaerales — Archaea
iii1-15
Acimicrobiales
Actinomycetales
0319-7L14
Gaiellales
Solirubrobacterales
Bacillales
Rhizobiales
Rhodospirillales
Burkholderiales
Myxococcales
Pseudomonadales
Xanthomonadales
Not cultivated
Not known to science
} Bacteria

representatives of the orders *Acimicrobiales*, *0319-7L14*, *iiil-15*, *Bacillales*, *Rhizobiales*, *Rhodospirillales*, *Xanthomonadales*, *Myxococcales* were also common (0.6—1.6%). The share of unclassified sequences was 3.8%, and not cultured on nutrient media — 7.8%.

At the family level, 167 taxa were identified, 14.3% of which belonged to unidentified sequences, and 4.7% did to representatives of families that do not grow on nutrient media. The most numerous in the number of attributed nucleic sequences were: *Alcaligenacea* (37.9%), *Pseudomonadaceae* (20.1%), *Gaiellaceae* (5.7%), *Nitrososphaeraceae* (4.2%), less common — *Solirubrobacteraceae*, *Micrococcaceae*, *Syntrophobacteraceae*, *Pseudonocardioidaceae*, *Nocardioidaceae*, *Intrasporangiaceae*, *Streptomycetaceae*, *Comamonadaceae* (0.5—1.0%), etc. (Table 3).

214 taxonomic units were identified at the genus level, 23.3% of which belong to unidentified sequences and 10.1% — to representatives that are not cultivated on nutrient media. Among the identified representatives, *Achromobacter* (31.5%) and *Pseudomonas* (19.9%) were dominant. The representatives of genera *Candidatus Nitrososphaera* (4.2%) were found among the subdominants. Less common genera were *Bacillus*, *Rubrobacter* (0.4%), *Streptomyces* (0.3%), *Pseudonocardia, Thermomonas, Aeromicrobium, Steroidobacter, Agromyces, Candidatus Solibacter* (0.2%), and *Nocardioides, Hyphomicrobium, Cellulomonas, Staphylococcus, Mycobacterium, Paenibacillus, Sporosarcina, Clostridium, Nitrospira, Alicyclobacillus, Sphingomonas, Kribbella, Nonomuraea, Rubrivivax, Arenimonas, Salinibacterium, Gemmata, Skermanella, Lamia, A4,*

Table 3. **Representation of the most common families
and genera of the prokaryotic complex of *Beta vulgaris* rhizosphere**

Family of microorganisms	Representation,%	Genera of microorganisms	Represen-tation,%
Alcaligenaceae	37.9	*Achromobacter*	31.5
		Uncultivated	0.4
		Unclassified	6.0
Pseudomonadaceae	20.1	*Pseudomonas*	19.9
		Unclassified	0.2
Nitrososphaeraceae	4.2	*Candidatus Nitrososphaera*	4.2
Sinobacteraceae	0.8	*Steroidobacter*	0.2
		Unclassified	0.4
		Uncultivated	0.2
Nocardioidaceae	0.6	*Aeromicrobium*	0.2
		Kribbella	0.1
		Nocardioides	0.1
		Unclassified	0.2
Pseudonocardioidaceae	0.6	Uncultivated	0.4
		Pseudonocardia	0.2
Streptomycetaceae	0.5	*Streptomyces*	0.3
		Nonomuraea	0.1
		Uncultivated	0.1
Comamonadaceae	0.5	*Rubrivivax*	0.1
		Uncultivated	0.4
Bacillaceae	0.4	*Bacillus*	0.4
Rubrobacteraceae	0.4	*Rubrobacter*	0.4
Hanthomonadaceae	0.4	*Thermomonas*	0.2
		Arenimonas	0.1
		Uncultivated	0.1
Hyphomicrobiaceae	0.4	*Rhodoplanes*	0.2
		Hyphomicrobium	0.1
		Unclassified	0.1
Microbacteriaceae	0.3	*Agromyces*	0.2
		Salinibacterium	0.1
Sphingomonadaceae	0.3	*Sphingomonas*	0.1
		Uncultivated	0.2
Solibacteraceae	0.2	*Candidatus Solibacter*	0.2
Chthonio-bacteraceae	0.2	*Candidatus Xiphinematobacter*	0.1
		Unclassified	0.1
Gemmataceae	0.2	*Gemmata*	0.1
		Unclassified	0.1
Rhodospirilaceae	0.2	*Skermanella*	0.1
		Unclassified	0.1

Continuation of table 3

Family of microorganisms	Representation,%	Genera of microorganisms	Represen-tation,%
Lamiaceae	0.1	*Lamia*	0.1
Cellulomonadaceae	0.1	*Cellulomonas*	0.1
Mycobacteriaceae	0.1	*Mycobacterium*	0.1
Flammeovirgaceae	0.1	*A4*	0.1
Caldilineaceae	0.1	*Caldilinea*	0.1
Alicyclobacillaceae	0.1	*Alicyclobacillus*	0.1
Paenibacillaceae	0.1	*Paenibacillus*	0.1
Planococcaceae	0.1	*Sporosarcina*	0.1
Staphylococaceae	0.1	*Staphylococcus*	0.1
Clostridiaceae	0.1	*Clostridium*	0.1
Nitrospiraceae	0.1	*Nitrospira*	0.1
Bradyrhizobiaceae	0.1	*Balneimonas*	0.1
Oxalobacteraceae	0.1	*Janthinobacterium*	0.1
Opitutaceae	0.1	*Opitutus*	0.1
Others *	9.5		
Others **	1.9	Others	6.1
Unclassified	14.3	Unclassified	16.1
Uncultivated ***	4.7	Uncultivated	8.3

Note: * *Gaiellaceae* (5.7), *Solirubrobacteraceae* (1.0), *Syntrophobacteraceae* (0.6), *Koll* 13, *Geodermatophilaceae*, *Patulibacteraceae* (0.3), *Enterobacteriaceae*, *Rhodobiaceae*, *Nocardiaceae* (0.2), *Frankiaceae*, *Conexibacteraceae*, *Chitinophagaceae*, *Rosieflexaceae*, *Ellin* 503, *Isosphaeraceae*, *Pirellulaceae*, *Burkholderiaceae*, *Haliangiaceae* (0.1), etc.; ** *Micrococcaceae* (1.0), *Intrasporangiaceae* (0.5), *Nocardiaceae*, *Micromonosporaceae*, *Solirubrobacteraceae*, *Enterobacteriaceae* (0.1) etc.; *** Uncultivated — microorganisms that are not cultivated on a nutrient medium.

Caldilinea, *Opitutus*, *Balneimonas* (0.1%), etc. (Table 3).

The genera *Achromobacter* and *Pseudomonas* were also found among the dominant microbiota, assessing the diversity of the soil microbial complex using classical microbiological methods, which was confirmed by the research results.

The species richness of the microorganisms of *Beta vulgaris* rhizosphere, according to the ecological Shannon (I_{Sh} = 5.36) and Simpson (I_S = 0.87) indices obtained by pyrosequencing were significantly higher than the results of classical microbiology. Therefore, the use of molecular biological research methods allows investiga-

tion to a greater extent of the microbial diversity structure, mainly due to uncultivated forms. The Chao saturation index (I_{Ch} = 1312.2) exceeds the number of detected operational taxonomic units by 6.1 times. That is, the level of real diversity of microorganisms in the culture rhizosphere was much higher than the experimentally detected metagenome of prokaryotes.

Discussion. In evaluating plant-microbial interactions in the rhizosphere, attention is paid not only to the quantitative accounting microorganisms but also to their qualitative composition. Despite the importance of classical microbiological methods for studying the qualitative

composition of soil microbes, they are not very informative in evaluating the genetic diversity of microorganisms because they can estimate only 0.1—10.0% of the soil microbial fund [8].

A complex combination of classical and molecular-genetics methods of analysis is relevant and necessary at present. The works of Rohandi [13], Andronov [14, 18], Kruglov [19], and Gorleknko [20] are devoted to studying the structure and biodiversity of the soil microbial complex by classical and molecular-biological methods. However, the data accumulated in this area are not sufficient. The pyrosequencing method used in the present work makes it possible to identify the real taxonomic diversity of the soil microbial complex, regardless of functional orientation, trophism, and cultivation on elective nutrient media, as well as to determine the quantitative indicators of certain taxa, including major and minor phyla of soil microbiota.

On the example of Beta vulgaris, it was shown that in the process of plant growth and development (including due to the root exudates intensity, uneven intake of easily digestible nutrients, and accumulation of plant residues in the soil), microbial diversity is formed in the culture rhizosphere and there is a redistribution of the structure of qualitative composition and diversity of microbial biome of the agroecosystem. The taxonomic structure of the sugar beet rhizosphere was determined by the pyrosequencing method. It included 15 phylas bacteria and 1 archaea. The dominant forms among the identified representatives were the phylas *Proteobacteria* (65.7%) and *Actinobacteria* (20.5%), and at the genus level — *Achromobacter* (31.5%) and *Pseudomonas* (19.9%). It should be noted that the results of our research coincide with the data of Andronov [14, 18], Chirak [21], Semenov [22], and Gorbacheva [23] who study microbiomes of different soil types by sequencing the libraries of the 16S-rRNA gene. These scientists found that the bacterial communities of soils are formed mainly by phyla *Acidobacteria, Actinobacteria,*

Bacteroidetes, Chloroflexi, Firmicutes, Gemmatimonadetes, Planctomycetes, Proteobacteria, and *Verrucomicrobia*, which are representatives of the cortical component of the soil microbiome. Dominant among them are *Proteobacteria* and *Actinobacteria*.

The soil microbial complex was established to be characterized by high biodiversity according to the indicators of ecological indexes determined on the basis of classical microbiological and molecular-biological research methods. Although the Shannon and Simpson indices determined by the pyrosequencing method, were much higher than ones determined by classical microbiological methods, the assessment of the microbial biodiversity of the sugar beet rhizosphere is consistent with the previously obtained data of Musilova [3], Melnichuk [24], Chernov [25], and Semenov [26]. They also found that the biodiversity of microorganisms varies depending on the soil type, moisture, organic matter content, and horizon depth. On average, the Shannon index varies between 4.3 and 7.5 for chornozem, according to the above scientists.

It should be noted that the results of our research are of great practical importance for the evaluation and optimization of new agricultural measures focused on increasing and disclosing the crop productive potential under the conditions of high-profitable production and maintaining soil fertility. In addition, the new knowledge of the taxonomic structure of the prokaryotes metagenome of chornozem typical in the sugar beet rhizosphere is of fundamental importance because it makes a significant contribution to the development of modern ideas of the formation of soil microbial biome.

Thus, the complex application of classical microbiological and molecular-biological methods of analysis allowed us to fully reveal the features of microbial metagenome formation of soil: structure, diversity, and taxonomic composition. Through the use of classical microbiological methods, it was found that the number of bacte-

ria and micromycetes increased during the ontogenesis of sugar beet plants due to the intensive production of root exudates by the plants. It is accompanied by redistribution in the structure of their morphotypes and increased diversity of microorganisms. The diversity of bacteria in the rhizosphere during plant ontogeny was higher by 7.9—38.8% compared with micromycetes and was characterized by a more uniform distribution and low dominance degree. The application of the pyrosequencing method allowed us to detect a high level of microbial diversity (I_{Sh} = 5.36) and to investigate it to a greater extent. 214 taxonomic units have been identified, of which 10.1% are forms that have not been cultivated on nutrient media and 23.3% are unclassified, but they are functionally significant for the rhizosphere. The most numerous at the family level were *Alcaligenaceae*, *Pseudomonadaceae*, *Gaiellaceae*, *Nitrososphaeraceae* and at at the genus level — *Achromobacter* and *Pseudomonas*.

Conclusions. During the ontogenesis of *Beta vulgaris*, including due to the intensive production of root exudates by the plants, the number of bacteria and micromycetes in the rhizosphere of plants increases. It is accompanied by redistribution of structural composition and an increase in the microorganisms' diversity (I_{Sh} = 5.36). It has been found that among the identified 214 taxonomic units, 10.1% are forms that are not cultivated on nutrient media, and 23.3% are unclassified. Our studies show that the structure of the microbial complex of the plants' rhizosphere reflects the characteristics of the soil and can be used as an indicator of its ecological status. The studies' results (obtained for the first time in the Forest-Steppe of Ukraine) deepen the knowledge of the true scale of natural genetic diversity of microbial complexes and are a valuable asset for substantiating practical proposals for effective adaptive interactions in the plant-microorganism system to preserve the homeostasis of agroecosystems.

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Ю.П. Борко¹, М.В. Патика², М.В. Бойко², А.М. Гончар², В.М. Сінченко³

¹ ННЦ «Інститут землеробства НААН України»,
  вул. Машинобудівників, 2-Б, Чабани, Фастівський р-н, Київська обл., 08162, Україна

² Національний університет біоресурсів і природокористування України,
  вул. Героїв оборони, 13, Київ, 03041, Україна

³ Інститут біоенергетичних культур та цукрових буряків НААН України,
  Клінічна вулиця, 25, Київ, 03141, Україна

ОСОБЛИВОСТІ ФОРМУВАННЯ ТАКСОНОМІЧНОЇ СТРУКТУРИ МІКРОБНОГО БІОМУ ГРУНТУ В РИЗОСФЕРІ *BETA VULGARIS*

Необхідність збільшення виробництва якісної сільськогосподарської продукції за умови мінімізації використання агрохімікатів зі збереженням високої рентабельності виробництва вимагає проведення комплексних досліджень визначального фактору ґрунтової родючості — його біологічної складової. Саме дослідження особливостей формування мікробіоценозів у ризосфері рослин на всіх стадіях онтогенезу дозволить розкрити механізми мікробно-рослинної взаємодії і розробити ефективні шляхи підвищення продуктивності сільськогосподарських культур на фоні високої функціональної активності та гомеостазу складової мікробного біому ґрунту. **Мета.** Дослідити класичними мікробіологічними і молекулярно-біологічними методами структуру мікробного комплексу та біорізноманіття ризосфери *Beta vulgaris* протягом онтогенезу. **Методи**. Чисельність мікроорганізмів визначали за методом посіву ґрунтових суспензій на агаризовані поживні середовища; структуру якісного складу мікроорганізмів — за морфологічно-культуральними властивостями; морфологію виділених ізолятів — за допомогою мікроскопіювання фіксованих препаратів. Різноманіття мікробних комплексів ґрунту оцінювали за екологічними індексами Шеннона, Сімпсона та Бергера-Паркера. Таксономічну структуру прокаріотів визначали за методом піросеквенування. **Результати**. Протягом онтогенезу Beta vulgaris за рахунок інтенсивності продукування рослинами кореневих ексудатів спостерігали диференціацію чисельності ґрунтової мікробіоти. Чисельність бактерій і мікроміцетів збільшувалась у 1—2.3 рази, проте у фазі змикання листків у міжрядді чисельність грибної мікробіоти зменшувалась на 46.4 %. Мікробні комплекси відрізнялися за кількістю виявлених морфотипів (27—50) і за структурою розподілу домінуючих форм (протягом вегетації культури загальна кількість домінуючих форм бактерій зменшувалась, а мікроміцетів — збільшувалась). Аналіз метагеному прокаріотів методом піросеквенування дав змогу виявити 214 операційних таксономічних одиниць, 10.1 % з яких — це форми, які не культивуються на поживних середовищах, а 23.3 % — не класифіковані. Серед виявлених таксономічних одиниць 96.2 % ідентифіковано на рівні порядку, 85.7 % — на рівні родини and 76.7 % — на рівні роду. Серед ідентифікованих таксонів 15 філ становили бактерії, 1 — археї. На рівні мікробних порядків виявлено 96 таксономічних одиниць, родин — 167, родів — 214. Домінуючими формами серед виявлених філ були представники *Proteobacteria* (65.7 %) та *Actinobacteria* (20.5 %); порядки — *Burkholderiales* (38.7 %) та *Pseudomonadales* (20.1 %); родини — *Alcaligenacea* (37.9 %), *Pseudomonadaceae* (20.1 %); *Gaiellaceae* (5.7 %), *Nitrososphaeraceae* (4.2 %); роди *Achromobacter* (31.5 %) та *Pseudomonas* (19.9 %). За показниками екологічних індексів, визначених на основі результатів класичних мікробіологічних і молекулярно-біологічних методів досліджень, встановлено, що мікробний комплекс ґрунту характеризувався високим біорізноманіттям, хоча показники індексу Шеннона ($I_Ш$ = 5.36) та Сімпсона (I_C = 0.87), визначені на основі результатів методу піросеквенування, були значно вищими аналогічних показників, визначених класичними мікробіологічними методами. **Висновки**. Протягом онтогенезу *Beta vulgaris*, у т. ч. завдяки інтенсивності продукування рослинами кореневих ексудатів, чисельність бактерій та мікроміцетів у ризосфері рослин збільшувалась. Це супроводжувалось перерозподілом структурного складу та збільшенням різноманіття мікроорганізмів ($I_ш$ = 5.36). Встановлено, що серед ідентифікованих 214 таксономічних одиниць 10.1 % — форми, що не культивуються на поживних середовищах і 23.3 % — некласифіковані. Проведені дослідження показали, що структура мікробного комплексу ризосфери рослин відображає особливості ґрунту і може бути використана як індикатор екологічного стану. Отримані результати (які проведені вперше в умовах Лісостепу України) поглиблюють знання щодо істинних масштабів природного генетичного різноманіття мікробних комплексів та є цінним надбанням для обґрунтування практичних пропозицій щодо формування ефективних адаптивних взаємодій у системі «рослина-мікроорганізми» з метою збереження гомеостазу агроекосистем.

Ключові слова: *метагеном, таксономічна структура, піросеквенування, морфотипи, ризосфера, буряк цукровий, онтогенез, індекси різноманіття.*