

# FORMATION OF THE EUBACTERIAL COMPLEX IN THE RHIZOSPHERE OF SUGAR BEET (*Beta vulgaris*) UNDER DIFFERENT FERTILIZATION SYSTEMS

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The aim of the work was to determine the quantitative, qualitative composition and taxonomic structure of the eubacterial complex in the rhizosphere of sugar beet under different fertilizer systems.

Microbiological methods were used to determine the content of microorganisms in the rhizosphere of sugar beet. Molecular methods were used to determine taxonomic structure as well as metagenome of the eubacterial complex of microorganisms.

In the agrocenosis of sugar beet under different fertilizer systems the representatives of such families as: *Alcaligenaceae*, *Pseudomonadaceae*, *Nitrososphaeraceae*, *Gaiellaceae*, *Micrococcaceae*, *Solirubrobacteraceae*, *Streptomyetaceae*, *Intrasporangiaceae*, *Solimonadaceae*, *Syntrophobacteraceae*, *Xanthomonadaceae*, *Enterobacteriaceae*, *Nocardioideae*, *Hyphomicrobiaceae*, *Comamonadaceae*. It was found that under the biological system of fertilizers the species diversity of soil microbiota increased due to phyla: *Alcaligenaceae*, *Gaiellaceae*, *Solirubrobacteraceae*, *Streptomyetaceae*, *Solimonadaceae*, *Syntrophobacteraceae*, *Xanthomonadaceae*, *Enterobacteriaceae*, *Nocardioideae*, *Hyphomicrobiaceae*, *Hyphomicrobiaceae*.

Besides, it was detected that the basis of the eubacterial complex of sugar beet included representatives of phyla *Proteobacteria*, *Actinobacteria*, *Gemmatimonadetes*, *Chloroflexi*, *Acidobacteria*, *Firmicutes*, *Planctomycetes*, *Verrucomicrobia*, *Bacteroidetes*, and the absolute dominants were *Proteobacteria* — 76.9%, *Actinobacteria* — 13.4%.

**Key words:** soil microbiota, fertilizer systems, metagenome, pyrosequencing, rhizosphere.

Soil microbiota is a determining factor in the processes of transformation and metabolism of nutrients, and therefore the formation of a high productivity of plants depends on its qualitative composition [1]. Accordingly, in soil agroecosystems, the ratio between the main phylum and families of soil microbiota is the main determinant of ecological balance and an indicator of effective formation of soil fertility [2]. The relevant data obtained by our scientists showed that the formation of soil was influenced significantly by the diversity of soil microorganisms, their structural organization and number [3]. This is why the presence of certain microbial populations in the soil may indicate the direction of the course of microbiological processes in it [4, 5]. Therefore, the homeostasis of agrocenosis directly depends

on the presence of soil microorganisms, which in turn can offset the impact of harmful anthropogenic load. Although in the process of interaction of excitatory factors of anthropogenic load, the microbiota in turn responds with changes in the dominant species of microorganisms. From the other hand, such changes in the microbiocenosis can serve as an indicator of the ecological state of the soil [6, 7].

Classical methods of studying the diversity of soil microbiota are widely used in microbiology, but they enable to identify only a slight range from 0.1 to 10% of the total representation for the soil microorganisms [8, 9]. However, the use of molecular biological methods to study the complex of soil microorganisms allows to determine the course of microbiological processes in multicomponent ecosystems, regardless of

the possibility to cultivate the certain species of microorganisms [10] and to quantify the prevalence of certain taxa [11].

### Materials and Methods

Studies of the eubacterial complex of the rhizosphere of sugar beet (*Beta vulgaris*) were conducted over 2016–2019 years at the Bila Tserkva research and the selection station of the Institute of Bioenergy Crops and Sugar Beet (IBC&SB) NAAS during the period of the active vegetation.

To identify the state of the soil microbiota and the course of the main microbiological processes, the generally accepted methods in soil microbiology were used [12, 13].

The microbiological analyzes were performed with the selection of 10 g of soil from each variant of the experiment. The experiments were performed in triplicate. The samples were transferred into sterile mortars and microorganisms were dispersed by the Zvyagintsev's method.

The number of microorganisms was determined by sowing the soil suspension on nutrient agar medium GPA. Establishment of the structure of microorganisms and their qualitative composition was performed according to morphological and cultural properties by microscopy of the fixed samples [14].

The scheme of the experiment on sugar beet fertilization provided application of different options for organic and mineral fertilizers treatment (Table 1).

The diversity of soil microbiota was determined by Shannon and Simson diversity indices and ChaoI saturation (comparison of the predicted number of operational taxonomic units (OTUs) by sampling parameters with the number of experimentally detected in the samples) [15].

For the analysis of taxonomic structure and metagenome, nucleic acids were isolated from soil microorganisms using the method of pyrosequencing with the following stages: creation of a library with fluorescent primers, double purification of PCR product, pyrosequencing, analysis of nucleotide sequence, determination of taxonomic structure and their comparative analysis [16].

Each experiment was tested in triplicate. Statistical analysis of experimental data was conducted by the method of analysis of variance using computer software Excel and Statistica — 10 [17].

### Results and Discussion

By studying the peculiarities of the formation of the eubacterial complex of sugar beet rhizosphere under the influence of different fertilizer systems in all variants of the experiment there were identified the most common representatives of phyla such as *Proteobacteria*, *Actinobacteria*, *Gemmatimonadetes*, *Chloroflexi*, *Acidobacteria*, *Firmicutes*, *Planctomycetes*, *Verrucomicrobia*, *Bacteroidetes*.

Subjecting to analysis the average representation of the identified phyla, the most common were the following: *Proteobacteria* — 76.9%, *Actinobacteria* — 13.4%, *Firmicutes* — 1.2%, *Acidobacteria* — 1.1%, *Gemmatimonadetes* — 0.8%, *Chloroflexi* — 0.8%, and the share of other phyla was 5.0% (Fig. 1).

The use of different fertilizer systems had an effect on the change in the ratio of different phyla representatives. Thus, among large phyla on the variants of the biological fertilizer system, the share of *Proteobacteria* increased to 78.7%, and the number of representatives of *Actinobacteria* increased to 14.5% compared to the control variant,

Table 1. Sugar beet fertilization system in short-rotation of crop rotation

No	System variant fertilization	Basic fertilizer	Pre-sowing fertilizer	Fertilizers for vegetative growth
1	Biological	Crop residues of wheat (8–10 t/ha) + Biohumus (vermicompost) «ECOCHUDO» 1 000 kg/ha	Seed treatment with biofertilizer Vermisol 10 l/t	Quantum-HUMAT, 0.7 l/ha
2	Ecological	Crop residues of wheat (8–10 t/ha) + N90P60K90	Seed treatment with biofertilizer Vermisol 10 l/t + for cultivation N55	–
3	Industrial	P90K120	Under cultivation N120P30K40	For revitalization N120P20K30

for which these indicators were 75.2 and 12.1% respectively. Similar growth results were observed in the variant of the ecological fertilizer system, which is most likely because of the activation of microorganisms responsible for the biological fixation of nitrogen and the decomposition of organic matter in the form of plant residues.

In the study of the metagenome of prokaryotes of the rhizosphere of sugar beet under different fertilizer variants, the dominance of such orders as Burkholderiales and Pseudomonadales was established. Differences in the structure of the dominant and subdominant orders depending on the system of sugar beet fertilization are shown in Fig. 2.

The application of biological and ecological fertilizer systems contributed to the increase in the number of representatives of the *Burkholderiales* order to 46.8% and 45.5%, while reducing the number of representatives of the *Pseudomonadales* order to 22.6 and 23.6%.

In general, subdominants include the following orders: *Gaiellales*, *Actinomycetales*, *Solirubrobacterales*, and *Acidimicrobiales*. The largest number among the subdominant was registered for representatives of *Solirubrobacterales* orders.

It was also found that the share of representatives of such orders as *Acidimicrobiales* and *Clostridiales* in the structure of soil microbiota was higher

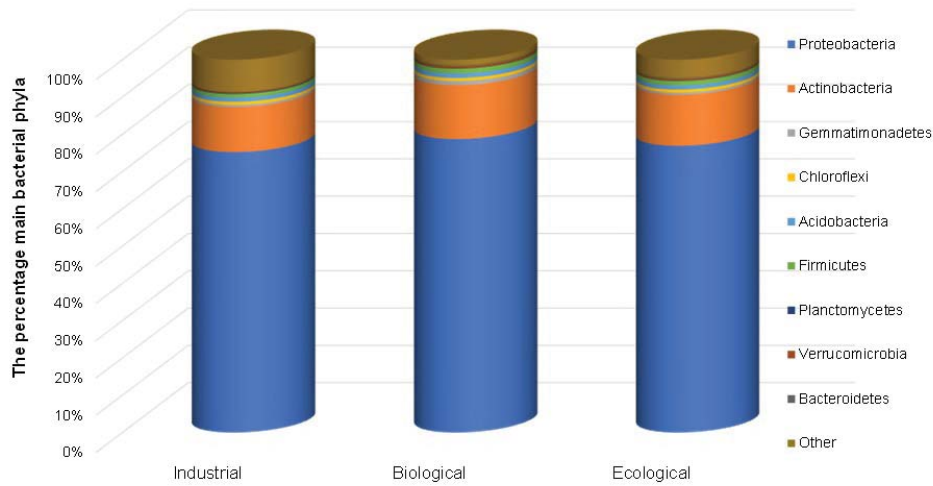


Fig. 1. Distribution of the main bacterial phyla of the microbial complex of the rhizosphere of sugar beet under different fertilizer systems

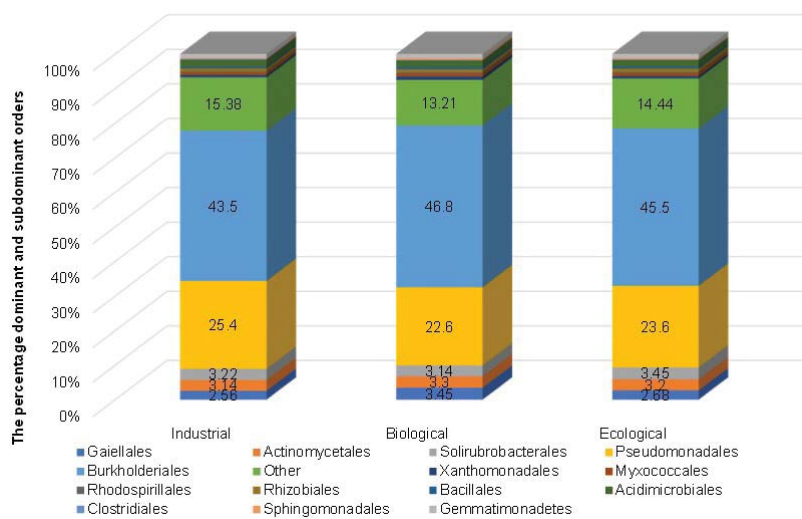


Fig. 2. Structure of dominant and subdominant orders of sugar beet rhizosphere prokaryotes under different fertilizer systems

when using the industrial system of sugar beet fertilization in comparison with the biological and ecological systems. The use of ecological and biological fertilizer systems has contributed to the growth of the number of representatives of such orders as *Actinomycetales*, *Xanthomonadales*, *Myxococcales*, *Rhodospirillales*, *Rhizobiales*, *Bacillales*, *Gaiellales*, and *Sphingomonadales*.

The results of the study of the prevalence of eubacterial complex families under different sugar beet fertilization systems are shown in Table 2.

It was established that such families as *Alcaligenaceae* and *Pseudomonadaceae* were dominant in sugar beet seedlings using different fertilizer variants. Moreover, under the industrial fertilizer system the share of members of the family *Pseudomonadaceae* was the highest, and with the use of biological and ecological systems it was decreased, while the share of members of the family *Alcaligenaceae* on the contrary was increased.

Accordingly, it can be affirmed that under the biological and ecological systems of fertilizers there was a decrease in the number of representatives of *Pseudomonadaceae* and *Nitrososphaeraceae* and an increase in the number of members of the families *Alcaligenaceae*, *Streptomyetaceae*, *Xanthomonadaceae*, *Enterobacteriaceae*, *Nocardioideaceae*.

According to the results of the research, the indices of diversity of the eubacterial complex under different sugar beet fertilization systems were calculated (Table 3).

It was determined that the ChaoI saturation index was greater than the number of identified operational taxonomic units (OTUs), and depending on the variant of the experiment ChaoI exceeded this indicator from 5.82 to 6.18 times. The identified differences between the ChaoI index and OTUs indicate really high levels of biodiversity of the eubacterial complex compared to the identified metagenome.

The greatest diversity of prokaryotes according to the Shannon index was in the biological fertilizer system — 4.98, and the least — in the industrial version of sugar beet fertilization. Thus, the use of organic fertilizers, compared with mineral ones, contributed to the formation of a variety of bacteria.

The investigations have shown that the basis of the eubacterial complex of sugar beet were representatives of the phyla *Proteobacteria*, *Actinobacteria*, *Gemmatimonadetes*, *Chloroflexi*, *Acidobacteria*, *Firmicutes*, *Planctomycetes*, *Verrucomicrobia*, *Bacteroidetes*. The absolute dominants were representatives of bacterial phyla *Proteobacteria* — 76.9%, *Actinobacteria* — 13.4%. The application of molecular methods of analysis enabled to determine that in the agrocenosis of sugar beet under different fertilizer

Table 2. Prevalence of eubacterial complex families under different sugar beet fertilization systems, %

Family	Industrial	Biological	Ecological
<i>Alcaligenaceae</i>	41.20	45.63	42.35
<i>Pseudomonadaceae</i>	32.80	24.56	31.00
<i>Nitrososphaeraceae</i>	3.02	2.83	2.99
<i>Gaiellaceae</i>	2.66	2.82	2.54
<i>Micrococcaceae</i>	1.45	1.40	1.47
<i>Solirubrobacteraceae</i>	0.91	1.29	0.84
<i>Streptomyetaceae</i>	7.05	7.23	7.11
<i>Intrasporangiaceae</i>	0.63	0.60	0.65
<i>Solimonadaceae</i>	0.42	0.61	0.33
<i>Syntrophobacteraceae</i>	0.40	0.58	0.24
<i>Xanthomonadaceae</i>	0.51	0.68	0.53
<i>Enterobacteriaceae</i>	0.31	0.43	0.35
<i>Nocardioideaceae</i>	0.28	0.64	0.55
<i>Hyphomicrobiaceae</i>	0.25	0.58	0.39
<i>Comamonadaceae</i>	0.17	0.76	0.39
Others	8.11	10.12	8.66

Table 3. Variety of eubacterial complex under different sugar beet fertilization systems

Fertilizer system	Number of OTUs	ChaoI Index	Shannon Index	Simson Index
Industrial	189	1 167.34	4.11	4.11
Biological	224	1 304.23	4.98	4.98
Ecological	205	1 248.66	4.43	4.43

systems, the predominant distribution included representatives from families *Alcaligenaceae*, *Pseudomonadaceae*, *Nitrososphaeraceae*, *Gaiellaceae*, *Micrococcaceae*, *Solirubrobacteraceae*, *Streptomycetaceae*, *Intrasporangiaceae*, *Solimonadaceae*, *Syntrophobacteraceae*, *Xanthomonadaceae*, *Enterobacteriaceae*, *Nocardioideaceae*, *Hyphomicrobiaceae*, *Comamonadaceae*.

The use of a biological fertilizer system is accompanied by an increase in the species diversity of soil microbiota due to phyla *Alcaligenaceae*, *Gaiellaceae*, *Solirubrobacteraceae*, *Streptomycetaceae*, *Solimonadaceae*, *Syntrophobacteraceae*,

*Xanthomonadaceae*, *Enterobacteriaceae*, *Nocardioideaceae*, *Hyphomicrobiaceae*, while the use of the ecological fertilizer system — due to such phyla: *Alcaligenaceae*, *Micrococcaceae*, *Streptomycetaceae*, *Intrasporangiaceae*, *Nocardioideaceae* and *Hyphomicrobiaceae*.

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Authors declare no conflict of interest.

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### ФОРМУВАННЯ ЕУБАКТЕРІАЛЬНОГО КОМПЛЕКСУ РИЗОСФЕРИ ЦУКРОВИХ БУРЯКІВ (*Beta vulgaris*) ЗА РІЗНИХ СИСТЕМ УДОБРЕННЯ

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Метою роботи було визначити кількісний та якісний склад і таксономічну структуру еубактеріального комплексу в ризосфері цукрових буряків з використанням різних систем удобрення.

Мікробіологічними методами визначали вміст у ризосфері цукрових буряків мікроорганізмів, а молекулярними — таксономічну структуру та метагеном еубактеріального комплексу мікроорганізмів.

В агроценозі цукрових буряків за різних систем удобрення переважне поширення мали представники родин *Alcaligenaceae*, *Pseudomonadaceae*, *Nitrososphaeraceae*, *Gaiellaceae*, *Micrococcaceae*, *Solirubrobacteraceae*, *Streptomyetaceae*, *Intrasporangiaceae*, *Solimonadaceae*, *Syntrophobacteraceae*, *Xanthomonadaceae*, *Enterobacteriaceae*, *Nocardioideae*, *Huiphomicrobiaceae*, *Cotamonadaceae*. Досліджено, що за біологічної системи удобрення збільшувалось видове різноманіття мікробіоти ґрунту за рахунок філ: *Alcaligenaceae*, *Gaiellaceae*, *Solirubrobacteraceae*, *Streptomyetaceae*, *Solimonadaceae*, *Syntrophobacteraceae*, *Xanthomonadaceae*, *Enterobacteriaceae*, *Nocardioideae*, *Huiphomicrobiaceae*, *Huiphomicrobiaceae*.

Встановлено, що основу еубактеріального комплексу цукрових буряків становили представники філ *Proteobacteria*, *Actinobacteria*, *Gemmatimonadetes*, *Chloroflexi*, *Acidobacteria*, *Firmicutes*, *Planctomycetes*, *Verrucomicrobia*, *Bacteroidetes*, а абсолютними домінантами були: *Proteobacteria* — 76,9%, *Actinobacteria* — 13,4%.

**Ключові слова:** ґрунтова мікробіота, системи удобрення, метагеном, піросеквенування, ризосфера.

### ФОРМИРОВАНИЕ ЭУБАКТЕРИАЛЬНОГО КОМПЛЕКСА РИЗОСФЕРЫ САХАРНОЙ СВЕКЛЫ (*Beta vulgaris*) ПРИ ПРИМЕНЕНИИ РАЗНЫХ СИСТЕМ УДОБРЕНИЯ

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Целью работы было определить количественный и качественный состав, а также таксономическую структуру эубактериального комплекса в ризосфере сахарной свеклы при использовании различных систем удобрения.

Микробиологическими методами определяли содержание в ризосфере сахарной свеклы микроорганизмов, а молекулярными — таксономическую структуру и метагеном эубактериального комплекса микроорганизмов.

В агроценозах сахарной свеклы при различных системах удобрения преимущественное распространение имели представители семей *Alcaligenaceae*, *Pseudomonadaceae*, *Nitrososphaeraceae*, *Gaiellaceae*, *Micrococcaceae*, *Solirubrobacteraceae*, *Streptomyetaceae*, *Intrasporangiaceae*, *Solimonadaceae*, *Syntrophobacteraceae*, *Xanthomonadaceae*, *Enterobacteriaceae*, *Nocardioideae*, *Huiphomicrobiaceae*, *Cotamonadaceae*. Доказано, что при биологической системе удобрения увеличивалось видовое разнообразие микробиоты почвы за счет фил: *Alcaligenaceae*, *Gaiellaceae*, *Solirubrobacteraceae*, *Streptomyetaceae*, *Solimonadaceae*, *Syntrophobacteraceae*, *Xanthomonadaceae*, *Enterobacteriaceae*, *Nocardioideae*, *Huiphomicrobiaceae*, *Huiphomicrobiaceae*.

Установлено, что основу эубактериального комплекса сахарной свеклы составляли представители фил *Proteobacteria*, *Actinobacteria*, *Gemmatimonadetes*, *Chloroflexi*, *Acidobacteria*, *Firmicutes*, *Planctomycetes*, *Verrucomicrobia*, *Bacteroidetes*, а абсолютными доминантами были: *Proteobacteria* — 76,9%, *Actinobacteria* — 13,4%.

**Ключевые слова:** почвенная микробиота, системы удобрения, метагеном, пиросеквенирование, ризосфера.