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

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Received 20.12.2020
Revised 15.02.2021
Accepted 27.01.2021

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, Streptomycetaceae, Intrasporangiaceae, Solimonadaceae, Syntrophobacteraceae, Xanthomonadaceae, Enterobacteriaceae, Nocardioidaceae, 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, Streptomycetaceae, Solimonadaceae, Syntrophobacteraceae, Xanthomonadaceae, Enterobacteriaceae, Nocardioidaceae, 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.
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 87.7%, 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) "ECOCUCHDO" 1 000 kg/ha | Seed treatment with biofertilizer Vermisol 101/t | Quantum-HUMAT, 0.7 l/ha |
| 2  | Ecological                    | Crop residues of wheat (8–10 t/ha) + N90P60K90 | Seed treatment with biofertilizer Vermisol 101/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.
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, Streptomyces, Xanthomonadaceae, Enterobacteriaceae, Nocardioidaceae.

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

| Family                  | Industrial | Biological | Ecological |
|-------------------------|------------|------------|------------|
| Alcaligenaceae          | 41.20      | 45.63      | 42.35      |
| Pseudomonadaceae        | 32.80      | 24.56      | 31.00      |
| Nitrososphaeraceae      | 3.02       | 2.83       | 2.99       |
| Gaiellaceae             | 2.66       | 2.82       | 2.54       |
| Micrococcaceae          | 1.45       | 1.40       | 1.47       |
| Solirubrobacteraceae    | 0.91       | 1.29       | 0.84       |
| Streptomyces             | 7.05       | 7.23       | 7.11       |
| Intrasporangiaceae      | 0.63       | 0.60       | 0.65       |
| Solimonadaceae          | 0.42       | 0.61       | 0.33       |
| Syntrophobacteraceae    | 0.40       | 0.58       | 0.24       |
| Xanthomonadaceae        | 0.51       | 0.68       | 0.53       |
| Enterobacteriaceae      | 0.31       | 0.43       | 0.35       |
| Nocardioidaceae         | 0.28       | 0.64       | 0.55       |
| Hyphomicrobiaceae       | 0.25       | 0.58       | 0.39       |
| Comamonadaceae          | 0.17       | 0.76       | 0.39       |
| Others                  | 8.11       | 10.12      | 8.66       |
systems, the predominant distribution included representatives from families Alcaligenaceae, Pseudomonadaceae, Nitrososphaeraceae, Gaiellaceae, Micrococcaceae, Solirubrobacteraceae, Streptomyctaceae, Intrasporangiaceae, Solimonadaceae, Syntrophobacteraceae, Xanthomonadaceae, Enterobacteriaceae, Nocardioidaceae, 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, Streptomyctaceae, Solimonadaceae, Syntrophobacteraceae, Xanthomonadaceae, Enterobacteriaceae, Nocardioidaceae, and Hyphomicrobiaceae. While the use of the ecological fertilizer system — due to such phyla: Alcaligenaceae, Micrococcaceae, Streptomyctaceae, Intrasporangiaceae, Nocardioidaceae, and Hyphomicrobiaceae.

The study was supported by a project funded by the Ministry of Education and Science of Ukraine (State Registration No.0116U002527).

Authors declare no conflict of interest.

### 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            | 1167.34     | 4.11          | 4.11         |
| Biological        | 224            | 1304.23     | 4.98          | 4.98         |
| Ecological        | 205            | 1248.66     | 4.43          | 4.43         |

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ФОРМУВАННЯ ЕУБАКТЕРИАЛЬНОГО КОМПЛЕКСУ РИЗОСФЕРИ ЦУКРОВИХ БУРЯКІВ (Beta vulgaris) ЗА РІЗНИХ СИСТЕМ УДОБРЕННЯ

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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 преимущество получали представители семейств Alcaligenaceae, Pseudomonadaceae, Nitrososphaeraceae, Gaiellaceae, Micrococcaceae, Solirubrobacteraceae, Streptomycetaceae, Intrasporangiaceae, Solimonadaceae, Enterobacteriaceae, Nocardioidaceae, Syntrophobacteraceae, Xanthomonadaceae, Enterobacteriaceae, Nocardioidaceae, Hyphomicrobiaceae, Hyphomicrobiaceae.

Установлено, что основу эубактериального комплекса сахарной свеклы составляли представители фил: Alcaligenaceae — 76,9%, Actinobacteria — 13,4%.

Ключові слова: грунтова мікробіота, системи удобрения, метагеном, піросеквенування, ризосфера.

ФОРМИРОВАНИЕ ЭУБАКТЕРИАЛЬНОГО КОМПЛЕКСА РИЗОСФЕРЫ САХАРНОЙ СВЕКЛЫ (Beta vulgaris) ПРИ ПРИМЕНЕНИИ РАЗНЫХ СИСТЕМ УДОБРЕННИЯ

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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 преимущественно распространение имели представители семейств Alcaligenaceae, Pseudomonadaceae, Nitrososphaeraceae, Gaiellaceae, Micrococcaceae, Solirubrobacteraceae, Streptomycetaceae, Intrasporangiaceae, Solimonadaceae, Enterobacteriaceae, Nocardioidaceae, Syntrophobacteraceae, Xanthomonadaceae, Enterobacteriaceae, Nocardioidaceae, Hyphomicrobiaceae, Hyphomicrobiaceae.

Установлено, что основу эубактериального комплекса сахарной свеклы составляли представители фил: Alcaligenaceae — 76,9%, Actinobacteria — 13,4%.

Ключевые слова: почвенная микрофлора, системы удобрения, метагеном, пиросеквенирование, ризосфера.