

THE FEATURES OF FORMATION OF MICROBIAL COMPLEX OF TYPICAL CHERNOZEM IN SUGAR BEET AGROCENOSISES

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The results of studies of qualitative and quantitative structure of the microbial complex which transforms the organic matter of typical chernozem at the sugar beets cultivation at the application of different agrarian systems are submitted. It is established that the application of biological agrarian system creates the optimal conditions for functional orientation of system soil - microorganisms - plant during the crop ontogeny.

Keywords: *microorganisms, sugar beets, typical chernozem, agrarian systems, soil tillage, metagenome*

Soil and formation of its structure are specified of the functions of complex biological systems which are complex polymorphic surroundings where the mealy biome of microorganisms existed and developed. Thus, according to modern scientific concepts, soil biota is a major foundation of genetic diversity in the world, much of which takes microorganisms [1, 5].

Pool of soil microbial complex makes important ecological functions. Investigation of the bioindication changes of these functions are evidence of the processes of soil formation, fertility, self-rectification capacity and cycling of substances [2]. The multifunctional soil processes, stable metabolic balance in ecosystems are provided with the microbiota [1, 5, 9]. Thus, microorganisms are very sensitive to changing of environmental conditions due to significant colonization of environment. The high rate of accumulation of biomass makes it possible to detect changes that occur under the influence of environmental factors in short term. This allows to predict the possible ways of changing of functional orientation of soil under the influence of agrarian systems and makes it possible evidence-based apply the

agrarian systems. It will ensure the preservation and restoration of soil fertility and high productivity of agro-ecosystems as a whole [9].

It should be noted that today, it is insufficiently covering the particularity of changing of qualitative and quantitative indicators of vital functions of microbial communities, despite the considerable attention of researchers to the diversity and functioning of the soil biocenosis [1, 2, 5, 9]. Therefore, the study of the structure and composition of soil microbial complex is essential aspect for understanding how soil environmental factors, soil properties, agrotechnical measures, different fertilization systems affect on biological processes.

The purpose of research – is research the qualitative and quantitative indexes of microbial complex of typical chernozem under different agrarian systems in sugar beet agroecosystem.

Materials and methods of research. The study of soil microbial communities are held at the stationary experiment of the department of agriculture and herbology of NULES of Ukraine «Agronomy Research Station» in the forest-steppe zone of the grain-beet crop rotation in 2012-2014. Soil samples was carried out from the upper arable horizon (0-20 cm) and sugar beet rhizosphere (*Beta vulgaris*) in the main phases of crop ontogenesis.

The scheme of experiment is provided the application of three agrarian systems (AS) against a background of two basic soil tillage (ST): 1) Industrial AS - (control) - (application of $N_{92}P_{100}K_{108}$, 12 tons of manure per hectare of crop rotation, intensive use of chemical plant protection products) + surface ST (cultivation of disk tools to a depth of 8-10 cm under all crops rotation); 2) industrial AS + differentiated ST - (carrying out of plowing on different depth by 6 times, surface tillage under winter wheat after peas and corn silage by 2 times and tillage under barley by 1 time per crop rotation; 3) ecological AS (application of $N_{46}P_{49}K_{55}$, 24 tons of organic fertilizer (12 tons of manure, 6 tons of non-commercial harvest (straw), 6 tons of green manure crop mass), the use of chemical and biological products by the criterion of ecological and economic threshold of harmful organisms) per hectare of crop rotation+ surface ST; 4) ecological AS + differentiated ST; 5) biological AS (24 tons of organic

fertilizer, the use of biological plant protection) + surface ST; 6) biological AS + differentiated ST [7].

The number of basic physiological groups of microorganisms were determined by the method of seeding of soil suspensions on solid culture media [3]. The study of morphotypes of selected isolates was conducted according to conventional methods of morphologically-cultural properties [8]. The microscopy of fixed preparations of dominant forms of microorganisms, previously isolated in pure culture, was conducted on the light microscope with zoom lens $\times 100$ by the using of immersion [6, 8]. Phylotype diversity of soil complex was determined by the molecular-biological method of pirosequencing [5, 10]. The diversity of soil microbial complexes was evaluated by the using of ecological characteristic: Simpson, Berger-Parker and Shannon indexes [4]. Statistical analysis of the results of research are carried out in MS Excel.

Results. It is revealed by the investigation of chernozem typical under sugar beet crops (in the rhizosphere and the arable layer) that the ratio and the number of different physiological groups of soil microorganisms depends on the phenophase, the quantity of applying organic and mineral fertilizers and the methods of soil tillage.

Thus, the number of soil microbiota in the shoots phase was the lowest and ranged within 4,7-16,8 million CFU / 1 g. a.d.s. (ammonifying, nitrifying, oligonitroifying, phosphate immobilizing, oligotrophic, pedotrophic microorganisms). The number of procaryotes is insreased in 1,1-2,4 (5,1-40 mln.) and 2,4-3,7 times (17,4-40 mln.) from the the middle to the end of vegetation. The quantitative of the streptomycetes was much lower (0,2-2 mln.) and did not change during the vegetation. The number of micromycetes (18,6-39,8 thousand) and cellulolitic microorganisms (14,3-81,3 ths.) was at the same level from the beginning to the middle of sugar beet vegetation. The number of micromycetes is increased in 1,2-3,4 times at the period of full maturity. The number of cellulolitic microorganismsis decreased in 2,1-4,2 times at the same time. It is creates favorable conditions for the optimal functioning of soil microbial cenosis (the numerical structure is insreased)

during crop ontogeny at the application of biological and ecological AS by the use of organic matter (manure, crop remnants, green manure).

The biogenic of soil microbial complex is increased during the vegetation and the highest it was at the end of its ontogeny. The total number of microbiota was higher in the rhizosphere than in the arable horizon by an average of 31,8 % (Fig. 1). In general, higher indexes of total microorganisms of typical chernozem are detected at the biological AS (141,2-455,4 ths. CFU / 1 g. a.d.s.) during the vegetation of sugar beet. At the application of ecological AS the number of microorganisms is decreased on 17,8 % and industrial AS – 18,6 %. The use of surface ST during the ontogenesis is promoted to increase the biogenic of soil microbial complex by an average of 10,7 % compared to differentiated ST.

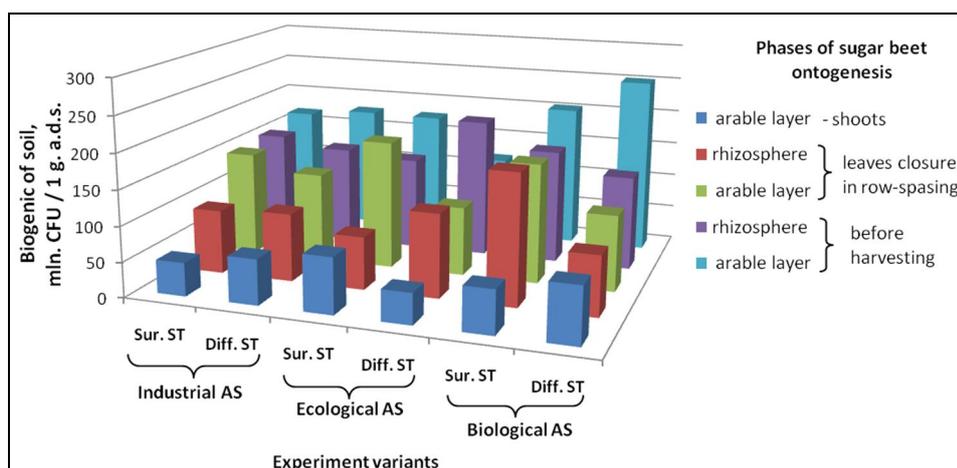


Fig. 1. The total number of microorganisms (biogenic) in typical chernozem

The analysis of the quality structure of microflora of typical chernozem is showed that the investigated variants are both differ in the number of identified morphotypes and the structure of distribution of dominant forms of microorganisms. Thus, at the phase of shoots, as throughout ontogeny, the greater diversity emerges at the application of biological and ecological AS. At the application of this AS the number of morphotypes of bacteria was higher by 27,5 and 24,5 % compared with the industrial AS, micromycetes by 61,8 and 50,0 %, respectively. In the phase of leaves closure in row-spasing the number of morphotypes is increased, but it was the

redistribution of microbial coenosis structure: the total number of morphotypes of bacteria at the biological AS is remained the largest (it is increased by 36,9 % compared with the beginning of vegetation) and micromycetes – is decreased (by 66,7 %). At the end of sugar beet ontogeny the quality variety of bacterial and fungal microflora was increasing at 4,2-40,0 % and 25,6-40,8 % respectively. It should be noted that the qualitative structure of bacteria morphotypes in typical chernozem was 2 times higher than the number of micromycetes morphotypes. The use of surface ST helped to increase the quality diversity of bacteria and micromycetes.

The distribution of the dominant members of microflora was uneven during the vegetation: the number of bacteria is increased and micromycetes – is decreased. It indicates on the formation of homeostatic microbial communities in typical chernozem.

It is established by the morphological characteristics that the dominant form of bacterial microflora are transparent and colored cocci (mostly single) and sticks (isolated, bacteria and bacillus that are placed in pairs and chains) of various sizes and shapes. Streptomycetes, who had filamentous form characteristic genera *Nocardia* and *Streptomyces*, are also often met (Fig. 2). The most common dominant morphotypes of bacterial microflora were representatives of: *Bacillus* (16-22 % of all AS), *Clostridium* (against a background surface ST: 12 % - industrial AS, 15 % - ecological AS, 17% - biological AS; 14 % - ecological AS + differentiated ST), *Micrococcus* (against a background surface ST: 16 % - industrial AS, 14 % - ecological AS), *Pseudomonas* (19-24 % at all AS), streptomycetes *Nocardia* (14 % - ecological AS + differentiated AS, 17 % - biological AS + surface ST).

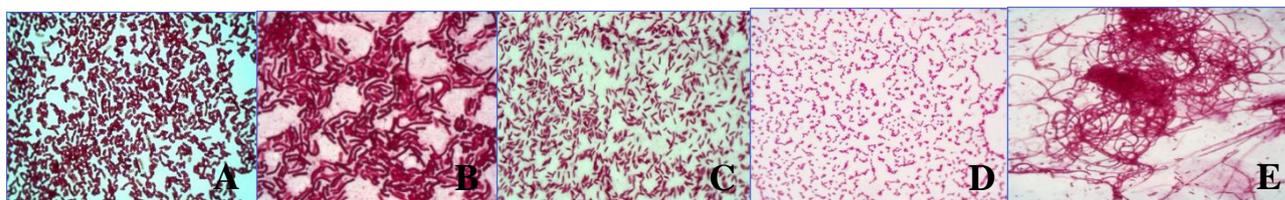


Fig. 2. The dominant morphotypes of bacterial microflora ($\times 100$):

A – *Bacillus*, B – *Clostridium*, C – *Micrococcus*, D – *Pseudomonas*, E - *Nocardia*

Analysis of microbial metagenome of typical chernozem at the domain level is showed that the absolute majority are bacteria. The researches of microbiome on the level of microbial sequences are showed the absolute dominance of *Burkholderiales* (38,7-45,7 %) and *Pseudomonadales* (20,1-31,4 %) with a slight variation in the studied agrarian systems. At the family level the distribution of the members of microbial cenosis at all experiment variants was characterized mainly a change in the ratio of major bacterial taxa. Among them the dominant were representatives of families *Alcaligenaceae* (37,9-44,8 %) and *Pseudomonadaceae* (20,1-34,1 %), subdominant - *Gaiellaceae* (2,3-5,7 %), *Nitrososphaeraceae* (2,4-4,2 %), the order *Solirubrobacterales* (family is not identified) (2,0-4,8 %), frequent - *Solirubrobacteraceae* (0,4-1,2 %), *Micrococcaceae* (0,5-1,0 %), the order *Rhodospirillales* (0,4-0,9 %) and others. (Fig. 3).

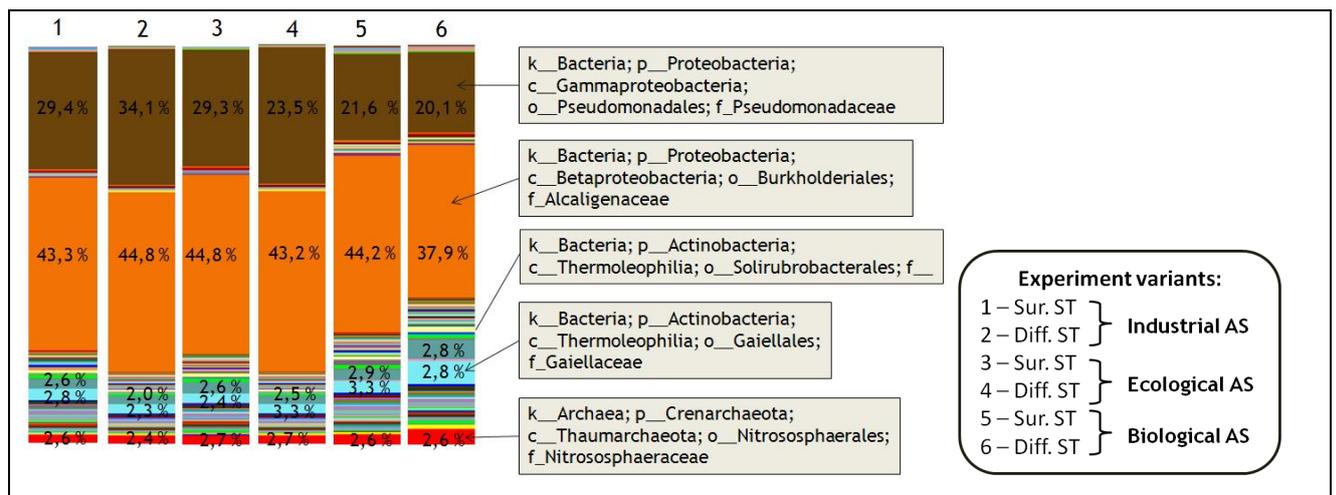


Fig. 3. Metagenome of prokaryotic complex of typical chernozem in sugar beet agrocenosis

It is revealed by the the Shannon' index that there are created more favorable conditions for the microbiota functioning at the biological AS in soil at the beginning and in the middle of the vegetation, as evidenced by higher biodiversity of microorganisms (on 1,2-20,5 % more than at the industrial AS). The application of surface ST is also promotes to increase the bacterial diversity on 5,2-6,0 % compared to differentiated ST, due to the localization of organic residues and fertilizers in the

upper soil layer. There were a clear inverse relationship between the Simpson and Shannon indexes, that indicates of existing system of microbial communities. The increasing of a Berger-Parker index, as a Simpson index, indicates to decrease of diversity and increase the degree of dominance of one species. Between the Berger-Parker index and Simpson ($r = + 0,8-0,93$), Shannon indexes ($r = -0,94$) was discovered as a close correlation, confirming preliminary findings. That is, the greatest diversity of bacterial and fungal microflora of the soil with the lowest degree of dominance of one species in the sugar beet agrocenosis are formed an the application of biological AS.

Conclusions

1. Agrarian measures and phenophases of sugar beet are significantly affected at the functioning of the microbial cenosis in the upper layer of typical chernozem and rhizosphere of culture, that appearing in the differentiation of their quantitative composition and structure.

2. The total number of microbiota was higher in the rhizosphere than in the arable horizon on 31,8%.

3. Surface ST promotes to increase the functional activity of microbiota by 10,7% compared with the differentiated ST. Application of industrial ST leads to decrease of number of microbial complex of typical chernozem by 18,6 % and biodiversity - by 27,5 % (compared with biological AS).

4. Biological AS (ecological AS somewhat lesser extent) promotes to increase the number on 17,8-18,6 % and biodiversity of microbiota on 1,2-20,5 % (compared with the industrial AS), expanding the trophic relationships of microbial communities and, ultimately, the formation of homeostatic microbial biomes of soil ecosystems.

Literature

1. Думова В. А. Изучение биоразнообразия комплекса прокариотных микроорганизмов подзолистых почв / В. А. Думова, Н. В. Патыка, Ю. В. Круглов и др. // Мікробіологія і біотехнологія. – 2009. - № 6. – С. 60-65
2. Марчик Т. П. Численность, биомасса и эколого-трофическая структура

- микробных ценозов дерново-карбонатных почв / Т. П. Марчик, С. Е. Головатый // Веснік Гродзенскага дзяржаўнага ўніверсітэта імя Янкі Купалы. - Серыя 5. Эканоміка. Сацыялогія. Біялогія. - № 1 (125). – 2012. – С. 107-118
3. Методы почвенной микробиологии и биохимии: Учеб. Пособие /Под ред. Д. Г. Звягинцева. – М.: Изд-во МГУ, 1991. – 304 с.
 4. Одум Ю. Основы экологии / под. ред. Н.П. Наумова. - М.: Мир, 1975. - 733 с.
 5. Патыка Н. В. Особенности филогенетических профилей прокариотических микроорганизмов подзолистых почв / Н. В. Патыка, Ю. В. Круглов, В. Ф. Патыка // Физиология и биохимия культурных растений. – 2009. – Т.41. - № 3. – С. 248-254
 6. Сэги Й. Методы почвенной микробиологии / Под. ред. Г. С. Муромцева, пер. с венгр. И. Ф. Куренного / Й. Сэги. – М.: Колос, 1983. – 296 с.
 7. Танчик С. П. Екологічна система землеробства в Лісостепу України. Методичні рекомендації для впровадження у виробництво /С. П. Танчик, О. А. Демідов, Ю. П. Манько. – К.: Видавничий центр НУБІП України, 2011. – 39 с.
 8. Теппер Е. З. Практикум по микробиологии / Е. З. Теппер, В. К. Шильникова, Г. И. Переверзева. — М.: Колос, 1972. – 239 с.
 9. Шерстобоева Е. В. Биоиндикация экологического состояния почв / Е. В. Шерстобоева, Я. В. Чабанюк, Л. И. Федак // Сільськогосподарська мікробіологія: міжвід. темат. наук. зб. – Чернігів. – 2008. – №. 7. – С.48-56
 10. Sequencing Method Manual for GS Junior Titanium Series [Текст] / Method Manual. – 454 Life Sciences Corp., A Roche Company Branford, 2012 – 26 p.

**Особенности формирования микробного комплекса чернозема типичного
в агроценозе свеклы сахарной**

Москалевская Ю. П., Патыка Н. В., Танчик С. П.

Представлены результаты исследований качественного и количественного состава микробного комплекса, который трансформирует

органическое вещество чернозема типичного при выращивании свеклы сахарной в различных системах земледелия. Установлено, что применение биологической системы земледелия создает оптимальные условия для функциональной направленности системы почва -микроорганизмы - растение в течении онтогенеза культуры

Ключевые слова: *микроорганизмы, свекла сахарная, чернозем типичный, система земледелия, обработка почвы, метагеном*

Особливості формування мікробного комплексу чорнозему типового в агроценозі буряка цукрового

Москалевська Ю. П., Патица Н. В., Танчик С. П.

Представлено результати досліджень якісного та кількісного складу мікробного комплексу, який трансформує органічні речовини чорнозему типового при вирощуванні буряка цукрового за різних систем землеробства. З'ясовано, що застосування біологічної системи землеробства створює оптимальні умови для функціональної спрямованості системи ґрунт–микроорганізми - рослина протягом онтогенезу культури

Ключові слова: *микроорганізми, буряк цукровий, чорнозем типовий, система землеробства, обробіток ґрунту, метагеном*