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Features of Changes in the Number and Composition of the Microbiome of the Rhizosphere of Winter Wheat in the Process of Ontogeny

Abstract. In agroecosystems, microorganisms are the main factor in the soil formation process, plant nutrition and phytosanitary condition of the soil. All measures aimed at restoring soil fertility and increasing productivity, environmental safety of agricultural production, are closely related to the activity of microorganisms. Increased local activity, biomass and diversity of microbiota are one of the most important characteristics that distinguish the rhizosphere from the total soil volume. The purpose of the research was to study the number and composition of the microbial complex of the rhizosphere of grain crops (winter wheat of different varieties of domestic selection) in the process of ontogenesis. To conduct a set of microbiological studies, soil samples (typical chernozem) were selected and prepared in compliance with standard requirements for sample preparation and

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storage in laboratory conditions. Analysis of rhizosphere soil samples was carried out on variants of different varieties of winter wheat of domestic selection. The number of soil microorganisms was determined by seeding suspensions on nutrient media according to generally accepted methods in microbiology. The determination of the total microbial biomass in the soil was carried out by the rehydration method, and the intensity of soil "breathing", in terms of CO_2 release and O_2 absorption, was determined by the manometric method (Warburg apparatus). It was found that winter wheat varieties affected the formation of the microbiome of typical chernozem. The number and composition of the microbial complex of the rhizosphere of winter wheat in the process of ontogenesis changes significantly, especially in terms of the ratio of the number of spore-forming and non-spore forms of microorganisms under the same conditions of agricultural technology for growing crops. The rhizosphere's total pool of saprotrophic microorganisms shows biomass variability and changes in favour of ecoplastic bacilli. An increase in the number of spore-forming bacteria to 4.2×10^7 CFU/g was found in the variants of cultivation of certain varieties. Cultivation of different varieties of winter wheat demonstrates stable indicators of CO_2 emission intensity – from 5.2 to 7.0. A similar trend can be traced in the absorption rate of O_2 (no more than 5.3-6.8)

Keywords: soil microorganisms, microbial biomass, varietal specificity, the intensity of CO_2 emission, dynamics of O_2 absorption, spore-forming bacteria

RELEVANCE

The microbiome is multifunctional and participates in soil formation, plant nutrition and determines the phytosanitary status of the soil. Microorganisms respond quickly to changes in environmental conditions and at a good ecological status of the soil is accompanied by restructuring in the microbial cenosis and its functional activity, and at a bad – leads to unpredictable consequences and death of certain groups of microorganisms. While adapting to new conditions of habitat, microbial cenosis shows resistance to them or irreversibly changes its functional properties, which affects soil fertility (Bulyhin, Tonkha, 2019). Agricultural technologies directly or indirectly affect the activity of microorganisms (Patyka, Tonkha *et al.*, 2019; Gadzalo, Patyka, Zarishnyak, 2015; 2019).

The diversity and structure of the bacterial complex in the rhizosphere is highly differentiated depending on the genotype of the plant, the type of soil, the level of agriculture, as well as the characteristics and morphology of the root system of plants (Berg, 2009). Thus, the level of genetic potential that determines the formation of plant-microbial systems, the effectiveness of symbiosis, the availability of nutrients and the likelihood of pathogenic infection depends on the plant variety, the group of rhizosphere microorganisms. The system of controlling the number of microorganisms is commonly known to be dominated by a specific microbiome that competes and exhibits biological activity (for example, antagonistic to plant pathogens). Thus, the ability of spore-forming bacteria of the genus *Bacillus* to neutralise phytopathogenic organisms may also be determined by both the high rate of occupation of their ecological niche in the rhizosphere and the biosynthesis of antibiotic substances and other antifungal metabolites (Olanrewaju, Glick et. al, 2017; Chebotar *et al.*, 2009).

The plant rhizosphere is a unique soil environment, the distinctive feature of which is the constant supply of low molecular weight compounds in the form of root exudates. A large amount of active microbiota is maintained in the rhizosphere, the biomass and polymorphism of which can be higher by several orders of magnitude than in the total arable soil layer.

Long-term experimental material on the diverse importance of soil microorganisms in the ontogeny of higher plants has been accumulated. The rhizosphere of plants is a promising environmental resource for the search for new universal agents of microbial preparations that create a powerful basis for high-tech biotechnological developments for the formation of plant-microbial interactions in agricultural crops. With the further development of comprehensive scientific research, the main focus is on both processes occurring in the rhizosphere and those related to human activity, especially agricultural production (Patyka, Tonkha *et al.*, 2018; 2019). The data on the specific features of changes in the number and composition of the microbiome of the rhizosphere of cereals (winter wheat) in the process of ontogeny should be expanded with regard to the available varieties of winter wheat.

ANALYSIS OF RECENT RESEARCH AND PUBLICATIONS

Today, the mechanisms of colonisation of plant organisms by microorganisms and the interaction of bacteria with them, which positively affect the growth and development of the plant, contribute to the process of mineral nutrition, protection against pathogens, etc. have already been established (Moshynets, Kosakivska, 2010). However, the rhizosphere microbe of cultivated plants is still insufficiently studied. At present, studies of the microbiome of the winter wheat rhizosphere, and the properties of the dominant species of soil microorganisms are of particular relevance. It is possible to thoroughly investigate the processes that occur in a particular environment (for example, the activity of mineralisation processes in the presence of spore bacteria or mineralisation of organic compounds, the availability of organic nitrogen, high enzymatic activity, changes in aeration conditions, etc.) Different variants of cereal crop cultivation and their varietal diversity reveal both quantitative differences in the representation of certain types of microorganisms and qualitative changes in the composition of the microbiome.

Increased local activity, biomass and diversity of microbiota are one of the most important characteristics that distinguish the rhizosphere from the total soil volume. The rhizosphere is characterised by a large number of nutrient substrates used by soil bacteria. Soil bacteria are able to competitively colonize plant roots and can be either biofertilisers or antagonists (the basis of microbial preparations) or both. Thus, the diversity of spore bacteria that dominate in agrocenoses directly or indirectly determines the processes of formation of productive biomass of cultivated plants – yield (Mandic-Mulec *et. al*, 2015; Patyka, Bublik et. al, 2015).

The soil prokaryotic complex is mutually beneficial for plants due to various mechanisms of interaction, in particular, through optimisation of nutrient supply to plants, antagonism to other microorganisms, especially pathogens, synthesis of growth regulators or enhancement of secondary metabolic pathways that are directly related to the increase of plant stress resistance (Smirnov, 2001). The study conducted by Gazheeva *et al.* (2011) revealed that the total number of rhizosphere microorganisms actively changes according to the phases of grain crop development (*Triticum aestivum* L., *Hordeum distichon* L., *Avena sativa* L.). There are certain uniform changes for barley, wheat in the tillering stage, where the total number of microorganisms is minimal and further reaches the maximum at the earing stage. The change in the number of bacterial communities of the rhizosphere during the growing season is primarily associated with changes in the composition and quantity of root exudates of plants, which are a source of nutrition for microorganisms.

The population of micromycete forms and their species structure of both rhizosphere and spikelet can correlate with the rate of development of the corresponding isogenic lines (for example, those determined by the dominant/recessive state of *VRN* genes). Therefore, there is an assumption that genes indirectly, through participation in the regulation of physiological and biochemical processes, can be involved in the formation and functioning of the mycocenosis of soft wheat (Mironovskaya 808 variety).

It is proved that virtually any agrotechnical technique is accompanied by a noticeable and statistically significant change in the structure of microbial communities, which in the future can be used to create new technologies for managing soil fertility and universal approaches for analysing the agroecological state of soils according to metagenomic analysis (Patyka *et al.*, 2016).

Long-term use of high doses of mineral fertilisers suppresses the development of nitrogen-fixing, cellulose-destroying, ammonifying microorganisms, while the activity of mineralisation and denitrification increases, which leads to the loss of soil humus and causes a decrease in its stability (Cerna *et al.*, 2003).

An important characteristic of the rhizosphere microbiome is the ratio of the main physiological groups of microorganisms. Research conducted by G.V. Dobrovolsky, V.P. Patyka, V.V. Volkogon, G.O. Iutynska, D.G. Tikhonenko, V.T. Yemtsev, K.B. Novosad, M.V. Patika and other scientists created the fundamental basis of the modern concept of soil microbiota. Today, the introduction of ecologically oriented t echnologies for growing crops requires a detailed study of the characteristics of the functioning of agrocenoses to establish the regularities of the process of forming the productivity of terrestrial phytocenosis and ensuring the sustainable development of the agricultural sector.

The purpose of the study is to identify the number and composition of the microbial complex of the rhizosphere of grain crops (winter wheat of different varieties of domestic selection) in the process of ontogenesis.

MATERIALS AND METHODS

The research was conducted on the basis of the National University of Life and Environmental Sciences of Ukraine, Prof. Shykula Department of Soil Science and Soil Protection and the separate subdivision of NUBiP of Ukraine "Agronomic Research Station" (Pshenychne, Vasylkiv district, Kyiv oblast), during the spring and summer vegetation of winter wheat (2019-2021). Soil type – typical deep low-humus coarse-dusted medium loam on loess-like loam, most common in the Right-Bank Forest-Steppe of Ukraine. To conduct a set of microbiological studies, soil samples (typical chernozem) were selected and prepared in compliance with standard requirements for sample preparation and storage in laboratory conditions, according to DSTU 4287 : 2004. Soil samples were examined in quadruplicate. Samples of rhizospheric soil were taken from the roots of plants after careful shaking, and in dynamics during the main phases of wheat organogenesis: emergence, earing, flowering and milky ripeness, repeated three times. The number of soil microorganisms was determined by sowing suspensions on nutrient media according to generally accepted methods in microbiology: GCU (Zvyagintseva), meat-peptone agar (MPA) to account for microorganisms that use organic forms of nitrogen; starch-ammonia agar (SAA) – for microorganisms that assimilate mainly nitrogen of mineral compounds; oligonitrophilic microorganisms on Ashby's medium; Chapek's medium and wort agar (WA) – for micromycetes; cellulose-degrading microorganisms – on Hutchinson's medium; in addition, bacteria growing on agarised soil extract (AS) were also taken into account. The degree of dominance of microorganisms above 10.0% was determined morphologically. The total microbial biomass in the soil was determined by the rehydration method (Blagodatsky, Blagodatskaya, Gorbenko, and Panikov, 1987). The intensity of soil "breathing" was determined as per CO_2 release and absorption of O_2 by the manometric method on the Warburg apparatus. Statistical analysis was performed using Statistica 8.0, and the data were calculated using MS Excel.

RESULTS AND DISCUSSION

The data on the number of soil microorganisms under winter wheat indicate the variability of microbial biomass by varietal variants. Thus, the number of spore-forming bacteria increased: in the varieties Trudivnytsia Myronivska, Manera Odeska, Liner, Legend Bilotserkivska and Poliska 90 was twice as high (4.2 x 10^7 CFU/g on average) compared to Mip Valencia, Mip Dnipryanka and other varieties of the Breeding and Genetic Institute of NAAS of Ukraine (2.0 x 10^7 CFU/g soil). This indicates the activation of bacillary forms of microorganisms during the ontogeny of wheat plants (Table 1, 1a).

Table 1. Microbiological analysis of samples taken from the rhizosphere of winter wheat of different varieties of domestic selection (SE NUBiP of Ukraine "Agronomic Research Station", 2019-2020)

Physiological groups m/o /nutrient medium/ quantitative unit	***Winter wheat varieties (Triticum aestivum* L.)**									
	Lainer (durum)	**Prozoryi (durum)**	**Shlyakhetnyy (durum)**	**Namysto**	**Shchedrivka Kyivska**	**Poliska 90 (etalon)**	**Lehenda Bilotserkivska**	**Lisova pisnya**	**Muza Bilotserkivska**	**Zdoba Kyivska**
m/o, (SAA), mln CFU/g	10.07± 0.29	10.27± 0.90	15.00± 1.03	10.27± 0.96	15.90± 0.87	11.70± 0.55	15.84± 0.90	11.70± 0.46	16.08± 1.03	12.18± 0.94
m/o, (SAA), mln CFU/g	15.03± 0.72	14.20± 0.37	11.93± 1.01	14.30± 0.41	12.30± 1.06	12.0± 1.02	13.90± 0.74	16.25± 0.88	14.52± 0.78	13.94± 0.75
Micromycetes thousand CFU/g	**52.30± 1.02**	48.5± 0.98	43.5± 1.02	45.8± 0.96	40.1± 0.87	**52.8 ±1.00**	**53.45 ±1.02**	44.6± 0.95	47.3± 0.67	48.6± 0.74
spore bacteria (LB), mln CFU/g	**3.72 ±1.03**	2.3± 0.67	1.99± 0.65	1.54± 0.98	1.65± 0.78	**4.20 ±0.98**	**4.0 ±1.06**	2.50± 0.98	1.59± 0.82	1.77± 0.92
Note: m/o – microorganisms; mln CFU/g of soil; (thousand CFU/g of soil);										

Table 1a. Microbiological analysis of samples taken from the rhizosphere of winter wheat of different varieties of domestic selection (SE NUBiP of Ukraine "Agronomic Research Station", 2019-2020)

Physiological groups m/o /nutrient medium/ quantitative unit	***Winter wheat varieties (Triticum aestivum* L.)**								
	MIP Valenciya	**MIP Dnipryanka**	**Trudovnitsya Myronovska**	**Myronovska Slava**	**Veteran**	**Harantiya Odeska**	**Manera Odeska**	**Nyva Odeska**	**Tradytsiya Odeska**
m/o, (SAA), mln CFU/g	15.27± 0.71	14.64± 0.33	16.13± 1.05	13.11± 0.16	20.97± 1.59	14.96± 1.09	16.77± 0.71	9.45± 0.58	8.60± 0.67
m/o, (SAA), mln CFU/g	14.56± 0.87	16.60± 0.98	13.19± 0.94	14.50± 0.78	15.38± 0.76	13.41± 0.79	11.99± 1.02	13.95± 0.74	16.60± 0.63
Micromycetes thousand CFU/g	47.60± 1.05	38.90± 1.20	43.0± 0.98	38.27± 0.95	40.9± 1.02	39.0± 0.93	51.2± 1.08	37.8± 0.88	39.9± 0.91
spore bacteria (LB), mln CFU/g	1.98± 0.68	1.67± 0.78	4.0± 1.05	2.0± 0.65	2.1± 0.86	1.88± 0.92	3.79± 0.82	1.66± 0.66	1.72± 0.83
Note: m/o – microorganisms; mln CFU/g of soil; (thousand CFU/g of soil);									

The development of freely existing forms of microorganisms of the genus *Azotobacter* was observed in the rhizosphere of winter wheat varieties. The number of micromycetes by varietal variants ranged from 38,270 to 53,450. CFU/g of soil.

The results of studies of the soil microbiome in the agrocenosis of winter wheat showed a significant differentiation in the number of microorganisms of the main physiological groups depending on the variety of plants (Table 2). The number of oligonitrophilic bacteria in the tilth layer of soil in winter wheat crops during the growing season increased and varied between 169-302 million CFU/g of soil (except for the variant with wheat variety MIP Dnipryanka), which indicates the passage of nitrogen-fixing processes. Nitrogen significantly affects the formation of plant productivity elements. Winter wheat is commonly known to have a long growing season, to bush up heavily in autumn and develop a powerful root system, to resume growth in early spring and assimilate a relatively large amount of nitrogen starting from the beginning of germination to the tube phase 75-90% of its total removal. Thus, the resumption of winter wheat vegetation in spring is accompanied by intensive growth processes, and therefore winter wheat plants are in urgent need of nutrients.

However, due to low temperatures and high soil moisture, which inhibit nitrification, the nitrogen content in the root layer in early spring is usually insufficient, which causes the onset of the first critical period in the nitrogen nutrition of plants in winter wheat. Observations of bacterial biomass (a living and sensitive component of organic matter) under different varieties of winter wheat reveal an increase in this indicator from 6 to 9 t/ha.

It is generally believed that the status of soil microbial communities depends on the farming systems (mineral or organic), and the agrotechnical methods used (fertiliser application, crop cultivation, crop rotation, plant residue management techniques). Thus, the variants of the experiment contain 75-132 million streptomycetes per 1 g. The relative quantity of mushrooms in the variants MIP Dnipryanka, Poliska 90, Namysto and Shchedrivka Kyivska was higher compared to other varietal variants (e.g. Garantiya Odeska, Lisova Pisnya, Trudivnytsya Myronivska, (Table 2).

Table 2. The number and biomass of soil microorganisms for the cultivation of different varieties of *Triticum* (2019-2021)

Variant	Biomass of bacteria, t/ha	Bacteria, on media			Oligonitrophilic bacteria	Micromycetes	Streptomycetes	cellulolytic thous./g of dry soil
		SAA	SAA+WA	AS				
		10^6 CFU/g of dry soil						
Namysto	6.9	11	6.0	136	169	3.2	75.0	28.6
Harantiya Odeska	7.7	27	8.1	179	192	2.9	89.3	26.1
Lisova pisnya	8.4	31	9.2	199	223	2.8	92.1	36.5
Trudovnitsya Myronovska	8.8	36	9.4	217	298	2.9	111.0	33.7
MIP Dnipryanka	6.9	21	7.3	154	1,871	3.6	98.0	22.7
MIP Valenciya	8.5	32	8.8	200	265	2.6	132.0	35.5
Poliska 90	5.7	14	5.8	127	176	3.4	87.0	26.4

Table 2, Continued

Variant	Biomass of bacteria, t/ha	Bacteria, on media			Oligonitrophilic bacteria	Micromycetes	Streptomycetes	cellulolytic thous./g of dry soil
		SAA	SAA+WA	AS				
		10^6 CFU/g of dry soil						
Shchedrivka Kyivska	6.6	19	6.4	135	183	3.0	79.2	27.5
Tradytsiya Odeska	7.1	24	7.4	165	193	2.7	90.4	30.2
Manera Odeska	9.1	39	9.1	231	302	2.5	101.2	34.6
Lainer	8.4	33	8.7	202	244	2.8	112.3	31.2
Valenciya	8.5	35	8.6	210	276	2.8	130.0	33.0
$LSD_{0,5}$	1.0	2.4	1.2	23.0	20.1	0.4	17.0	2.7

The number of bacteria on different nutrient media (SAA, AS, SAA +WA) was not stable and varied in the range from 5.8 to 231 x 10^6 CFU/g of dry soil.

The results of the research revealed that the cultivation of different varieties of winter wheat demonstrates stable indicators of CO_2 emission intensity – from 5.2 to 7.0. Similarly, the dynamics of O_2 absorption did not exceed 5.3-6.8 (Table 3). This proves that the experiment soil creates optimal conditions for the life of microorganisms, thereby increasing its biological activity.

Table 3. The intensity of CO_2 emission and O_2 absorption in the soil during wheat cultivation using various agrotechnologies

Variant	The intensity of CO_2 emission and O_2 absorption, µg/h	
	CO_2	O_2
Namysto	5.7	5.5
Harantiya Odeska	6.4	6.4
Lisova pisnya	5.9	5.7
Trudovnitsya Myronovska	6.0	5.9
MIP Dnipryanka	5.9	5.9
MIP Valenciya	6.1	6.0
Poliska 90	5.2	5.3
Shchedrivka Kyivska	5.6	5.5
Tradytsiya Odeska	6.0	5.9
Manera Odeska	7.0	6.8
Lainer	6.7	6.6
Valenciya	6.7	6.5

Note: x/P = 0.05; t_{st} = 3.11

Thus, by analysing the number of microorganisms of the rhizosphere of winter wheat, it was determined that varietal specificity had a significant impact on the formation of the microbiome in different phases of plant growth and development, which is obviously an integral indicator of the functional and metabolic activity of soil microorganisms.

It was established that the number and composition of the microbial complex of the winter wheat rhizosphere in the process of ontogeny significantly changes, especially in the ratio of the number of spore-forming and non-spore forms of microorganisms under the same conditions of agricultural technology of crop cultivation. The general pool of saprotrophic microorganisms of the rhizosphere is changing in favour of eco-plastic bacilli, therefore, the exploratory research of bioagents of the genus *Bacillus* is relevant for agricultural science and practices. Further comprehensive examinations will be carried out through the prism of assessing the diversity of isolates based on the results of microbiological, physiological, biochemical, molecular biological research methods.

CONCLUSIONS

The results of microbiological studies to determine the number and composition of the microbiome of winter wheat revealed the variability of microbial biomass by varietal variants. The activation of bacillary forms of microorganisms during plant ontogeny was established (increase in the number of spore-forming bacteria to 4.2×10^7 CFU/g in the variants of cultivation of individual varieties). Significant differentiation is observed in the analysis of the number of microorganisms of the main physiological groups depending on the variety of plants. Thus, the development of freely existing forms of microorganisms of the genus *Azotobacter* was detected. The number of micromycetes by varietal variants ranged from 38,270 to 53,450. CFU/g of soil. And the number of oligonitrophilic bacteria in the tilth layer of the soil in winter wheat crops during the growing season increased to 169-302 million CFU / g of soil, evidencing the ongoing nitrogen-fixation processes. The index of bacterial biomass under different varieties of winter wheat demonstrated a stable growth from 6 to 9 t/ha. At the same time, the number of bacteria on different nutrient media (SAA, AS, SAA +WA) was not stable and varied in the range from 5.8 to 231×10^6 CFU/g of dry soil. It was found that the cultivation of different varieties of winter wheat demonstrates stable indicators of CO_2 emission intensity – from 5.2 to 7.0. Similar results were observed for the process of O_2 absorption (less than 5.3-6.8).

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Особливості змін чисельності та складу мікробіому ризосфери озимої пшениці в процесі онтогенезу

Анотація. В агроекосистемах мікроорганізми є основним чинником ґрунтоутворення, живлення рослин та фітосанітарного стану ґрунту. Усі заходи, спрямовані на відновлення родючості ґрунту та підвищення продуктивності, екологічної безпеки сільськогосподарського виробництва, тісно пов'язані з діяльністю мікроорганізмів. Підвищена локальна активність, біомаса та різноманітність мікробіоти є одними з найважливіших характеристик, які відрізняють ризосферу від загального об'єму ґрунту. Метою досліджень було вивчення чисельності та складу мікробного комплексу ризосфери зернових культур (озимої пшениці різних сортів вітчизняної селекції) в процесі онтогенезу. Для проведення комплексу мікробіологічних досліджень були відібрані зразки ґрунту (чорнозем типовий), які були підготовлені з дотриманням стандартних вимог до пробопідготовки та зберігання зразків у лабораторних умовах. Аналіз ризосферних зразків ґрунту проводили на варіантах різних сортів озимої пшениці вітчизняної селекції. Чисельність ґрунтових мікроорганізмів визначали методом висіву суспензій на поживні середовища за загальноприйнятими в мікробіології методиками. Визначення загальної мікробної біомаси в ґрунті проводили регідратаційним методом, а інтенсивність "дихання" ґрунту за виділенням CO_2 та поглинанням O_2 визначали манометричним методом (апарат Варбурга). Встановлено, що сорти озимої пшениці впливали на формування мікробіому чорнозему типового. Чисельність і склад мікробного комплексу ризосфери пшениці озимої в процесі онтогенезу суттєво змінюється, особливо за співвідношенням кількості спороутворюючих і неспорових форм мікроорганізмів за однакових умов агротехніки вирощування культури. Загальний пул сапротрофних мікроорганізмів ризосфери демонструє варіабельність біомаси та зміни на користь екопластичних бацил. Виявлено збільшення кількості спороутворюючих бактерій до 4,2 х 107 КУО/г у варіантах вирощування окремих сортів. Вирощування різних сортів пшениці озимої демонструє стабільні показники інтенсивності емісії CO_2 - від 5,2 до 7,0. Аналогічна тенденція простежується і за коефіцієнтом поглинання O_2 (не більше 5,3-6,8)

Ключові слова: ґрунтові мікроорганізми, мікробна біомаса, сортова специфічність, інтенсивність емісії CO_2, динаміка поглинання O_2, спороутворюючі бактерії