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PROPERTIES OF MICROORGANISMS ISOLATED FROM SOILS UNDER CONVENTIONAL AND ORGANIC FARMING

*Objective. The article presents the results of research aimed at determining the influence of different methods of tillage on the functional diversity of the soil microbiota. Soil samples containing plant residues from agricultural plots under conventional and organic farming in the Kyiv oblast were used for the study. **Methods.** Analysis of soil microbiota using differential diagnostic nutrient media by serial dilutions of soil suspension was performed. To quantify the phosphate-mobilizing properties of the isolated microorganisms, the concentration of phosphorus in the solution was measured (grown in NBRIP liquid medium) and detected by the Arenius spectrophotometric method on a Ulab 102UV Spectrophotometer. **Results.** The soil of the plots under organic agrotechnology of cultivation was marked by a greater number of microorganisms of all ecological and trophic groups, except oligonitrophilic and phosphate-solubilizing bacteria. The vast majority of phosphate-transforming bacteria were isolated from the soil of agricultural plots under convection farming. The largest number of cellulose-degrading isolates was isolated from the soil under organic farming plots. Five isolates have the widest range of agronomically useful properties, in particular, the ability to mobilize organic and inorganic phosphates and cellulosolytic activity: 6b, 13b, 18b, 19b, and 8m. After incubation of the isolates on an NBRIP medium at 28 °C and 200 rpm for 72 hr, special analyzes for dissolved phosphorus content and pH level in the culture fluid were performed. Isolate 8m selected from chornozem (black soil) under convection agriculture and classified by us as Trichoderma sp. exhibited the highest phosphate-mobilizing activity. The vast majority of bacteria capable of phosphate transformation were isolated from the soil of agricultural areas affected by convection agriculture; and isolates capable of dissolving cellulose — from the soil of organic farming. **Conclusions.** The initial identification of certain isolates allowed us to classify them as Bacillus and Trichoderma. These isolates are important for further research with the prospect of creating a complex biological preparation with fungicidal properties and the ability to mobilize organic and inorganic phosphorus compounds.*

Keywords: *phosphate-solubilizing microorganisms, agronomically valuable microorganisms, cellulolytic microorganisms, chornozem (black soil).*

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Microorganisms are an extremely important factor in the formation of soil fertility. The presence of various groups of microorganisms in soil ecosystems determines their great importance in the processes occurring in the soil [1]. It is known that the main internal factor of changes in the taxonomic and functional diversity of microbiota in the soil is, in fact, the vital activity of microorganisms, which causes qualitative changes in soil organic matter through metabolic products, contributing to the restructuring of the microbial complex [2]. However, the use of fertilizers and preparations for treating seeds and different methods of tillage always change the conditions in which microorganisms live in the soil and, accordingly, their quantitative composition. The microbial community performs the function of maintaining soil homeostasis and thus quickly responds to changes in the environment. Therefore, microorganisms are bioindicators that provide information not only about the degree of soil degradation but also about the ecological status of the studied ecosystem in general [3]. The significant restructuring of soil microbiocenoses is accompanied by a decrease in their taxonomic diversity and the appearance of new dominants. Microbiological indicators are widely used in soil monitoring under conditions of prolonged anthropogenic load [4]. These indicators make it possible to assess the direction of the transformation of substances in soil conditions and to determine the depth of influence of one factor or another on the soil yet at early stages, which will help to prevent irreversible soil degradation in time.

According to the requirements of convection technologies for growing crops, seed treatment with various pesticides is a mandatory element in the technology of growing any crop. However, pesticides can pose a serious threat to the soil environment and human health, since many of their derivatives remain in the soil system for a significant period [5].

It is known that studies of the effect of various preparations on soil microbial communities are especially important for the approval of pesticides because their effect on soil microorganisms is not easy to predict. There is a huge amount of data on the opposite effect of the same substance on different groups of bacteria in different types of soils. Some pesticides stimulate the growth of microorganisms, while others inhibit or do not affect them at all [6, 7]. Therefore, it should be noted that, despite the considerable attention of researchers to the diversity and functioning of soil microbiocenoses, the issue of their changes in conditions of intensive agriculture has not yet been sufficiently highlighted. Their changes under conditions of intensive agriculture are still insufficiently covered. And the question of the agronomically useful properties of microorganisms inhabiting the soil under convection farming remains unanswered at all.

Materials and methods. Soil samples were taken during a three-year experiment from plots under intensive and organic farming of the Skvyrsky district of the Kyiv oblast using the generally accepted envelope method [8, 9]. From the plot of 25 m^2, five soil samples weighing 200 g were selected with a sterile spatula from a depth of to 20 cm. The samples weighing 1 kg were placed in a sterile bag from each variant. Then they were mixed, sieved, dried, and an average sample weighing 500 g was taken. The research was performed within the following 3—6 hr. It was turned out to be a typical deep low-humus coarse silty loamy chornozem (black soil). Wheat and corn had been grown on both types of soil over the last five years.

An intensive farming technology consisted in the classic tillage of the soil and the application of pre-emergence and post-emergence herbicides (of chloroacetamides and triazines groups along with the treatment of seeds with disinfectants.

An organic farming means minimal tillage and no pesticide application over the past five years, certified for the production of organic products by the certification body LLC «Organic Standard» on the basis of the Skvyrsky department of organic agricultural technologies of the Institute of Agroecology and Environmental Management of the NAAS in 2019—2020. The soil was a typical black soil with a humus content of 4.3%, characterized by the following nutrient content: easily hydrolyzed nitrogen (according to Cornfield) 11 mg/100 g, phosphorus (according to Chirikov) 24 mg/100 g, potassium (according to Chirikov) 8.5 mg/100 g. The reaction of the soil solution was 6.5 (neutral).

Soil microbiota was analyzed using differential diagnostic nutrient media by serial dilutions of soil suspension. To determine the amount of ammonificating and spore-forming bacteria, meat-peptone agar (MPA) was used, streptomycetes and microorganisms using mineral forms of nitrogen — starch-ammonium agar (SAA), microscopic fungi — Sabouraud's dextrose agar (SDA), oligotrophic — water agar (WA), oligonitrophilic — Ashby's medium, pedotrophic — soil extract medium (SEM). Colonies were counted, and the morphological and cultural properties of the selected isolates were studied by standard microbiological methods [10]. The quantity of microorganisms was expressed in the number of colony-forming units (CFU) per gram of absolutely dry soil, taking into account the moisture coefficient and dilution of the soil suspension.

For the growing up microorganisms universal media were used: MPA for bacteria and Czapek`s medium for micromycetes [11].

The ability of microorganisms to transform organic phosphorus-containing compounds was determined on Menkina's agar medium (g/L): $(NH_4)_2SO_4$ — 0.5; NaCl — 0.34; KCl — 0.3; $MgSO_4 \times 7H_2O$ — 0.3; $FeSO_4$ as trace element, $MnSO_4$ as trace element, $CaCO_3$ — 5.0; glucose — 10.0; nucleic acid (or lecithin) — 5.0; phytic acid — 1; agar-agar — 20.0.

The ability of microorganisms to transform inorganic sparingly soluble phosphates was studied on Pikovska's agar medium (g/L): glucose — 20.0; NaCl — 0.2; $Ca_3(PO_4)_2$ — 5.0; $MgSO_4$ — 0.1; $MgSO_4$ as a trace element; $FeSO_4$ as trace element; agar-agar —20.0.

The cellulosolytic ability of microorganisms was investigated on Hutchinson agar medium (g/L): KH_2SO_4 — 0.1; NaCI — 0.1; $CaCl_2$ — 0.1; $FeCI_3$ — 0.1; $MgSO_4 \times 7H_2O$ — 0.3; $NaNO_3$ — 2.5; agar-agar — 20.0; using plates of filter paper or adding carboxymethyl cellulose (CMC).

To quantitatively evaluate the phosphate-mobilizing properties of the isolated microorganisms, we measured the phosphorus concentration in solution (grown in a liquid medium NBRIP (National Botanical Research Institute's Phosphate), (g/L): glucose — 10.0; $Ca_3(PO_4)_2$ — 5.0; $MgCl_2{\cdot}H_2O$ — 5.0; $MgSO_4 \times 7H_2O$ — 0.25; KCl — 2.0; $(NH_4)_2SO_4$ — 0.1; pH 6.8—7.0 and detected by the Arenius method on a spectrophotometer Ulab 102UV Spectrophotometer [12—14]. After incubation of the isolates for 72 hr at 28 °C and 200 rpm, an analysis was performed for the content of dissolved phosphorus and the pH level in the liquid culture.

Physiological and biochemical characteristics of bacterial isolates were investigated in accordance with the methods described in Bergey's manual of systematic bacteriology [15].

Preliminary identification of the selected isolates was performed using express bacterial identification systems API KIT® (bioMérieux, France) based on the manufacturer's instructions. Commercial API ID32C and API 50CHB kits with introduced isolates were incubated for 48 hr at 28 ± 2 °C. The obtained results (API codes) were compared with the profiles of the Internet database of the corresponding set (API database).

Statistical calculations were performed using the standard computer programs Statistica 8.0 and Microsoft Excel 2010.

Results. Microbiological analysis of soils showed that under the conditions of long-term influence of intensive agriculture, there is observed the restructuring of their microbial group. The microorganisms isolated from typical deep low-humus coarse silty loamy black soil (chornozem) were characterized by the following functional diversity (Table 1).

The soil from the plots under organic agricultural technology was characterized by a large number of microorganisms of almost all taxonomic and ecological-trophic groups, with the exception of oligonitrophilic bacteria and phosphate mobilizing bacteria.

The number of ammonificating microorganisms from organic farming plots was almost 10 times higher than their quantity on the soils under intensive farming, while the number of micromycetes, streptomycetes, and bacteria using mineral nitrogen was practically at the same level.

In the published materials on gray forest soil, it was shown that under conditions of soil contamination with salts of various metals, the number of ammonificating bacteria increases by 26—31% compared to the control, and significant gradual increase in the number of micromycetes, streptomycetes, oligonitrophils, and pedotrophic bacteria was compared to the control [4]. Such changes indicate an improvement due to the trophic regime and activation of mineralization processes [16].

It should also be noted that the microbiota of the soil sampled in the field under intensive farming was characterized by great diversity and predominance of pigmented forms of bacteria in comparison with the bacterial microbiota of the field of organic farming. It is known that one of the universal mechanisms of adaptation, for example, to toxic forms of photosensitized oxygen is the synthesis of carotenoid pigments [17]. Likewise, pesticides can affect the biological functions of microorganisms, their diversity, composition, and biochemical processes. They can often cause an imbalance between the functioning of the microbiome and soil fertility leading to crop losses [5, 18].

For a comparative study on the number of agronomic beneficial microorganisms in the studied soils, the most typical isolates with the ability to mobilize organic and inorganic forms of phosphates or to destroy cellulose were selected. In total, 24 bacteria and 12 micromycetes were isolated. For the convenience of work, bacterial isolates were marked with the letter «b», and micromycete isolates — letter «m». They were dispense on plates with differential media such as Menkina`s and Pikovska`s (for phosphatemobilising bacteria) and Hutchinson and Czapek's ones with the addition of filter paper or CMC (for cellulosolitics). This allowed us to quickly and efficiently identify microorganisms with such agronomically useful properties as the ability to mobilize organic and inorganic forms of phosphates and to degrade cellulose (Fig. 1).

In general, the highest phosphate-mobilizing activity was shown by 9 bacterial isolates and 5 micromycete isolates from the soil under convection farming. 6 bacterial isolates and 5 micromycetes isolated from the soil under organic

Table 1. **The number of microorganisms in the plant rhizosphere**

Variant	Microorganisms, CFU/g of soil						
	Ammonificating bacteria, 10^6	Phosphate-solubilizing bacteria, 10^6	Micromycetes, 10^3	Cellulose-destroying, 10^5	Streptomyces, 10^6	Oligonitrotrophic bacteria, 10^6	Bacteria using mineral forms of nitrogen, 10^6
Organic farming field	136.8 ± 18.3	26.2 ± 5.2	2.8 ± 0.4	5.4 ± 0.6	1.5 ± 0.04	1.2 ± 0.03	2.8 ± 0.05
Intensive farming field	14.1 ± 3.8	37.4 ± 9.4	2.4 ± 0.2	4.2 ± 0.3	1.1 ± 0.05	7.8 ± 0.1	2.3 ± 0.05

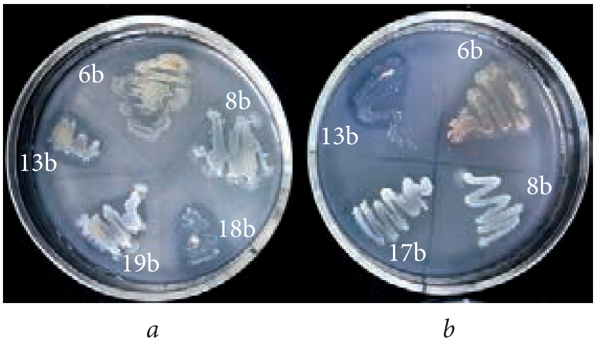

Fig. 1. Formation of transparent zones on selected media by some of the selected bacterial isolates: *a* —Pikovska's medium *b* — Menkina's medium

farming exhibited the highest phosphate-solubilizing activity. These isolates were further analyzed in detail.

No microorganisms with high cellulolytic activity were detected in the experimental agricultural plots. In general, isolates 6b, 13b, 18b, 19b, and 8m have the widest range of the following agronomically useful properties (Table 2).

Table 2 show that solubilizing organic phosphates microorganisms, which require the inclusion of more complex mechanisms than microorganisms converting only inorganic forms of phosphorus, dominate in the soils under convection farming. This is mainly due to the constant application of a large number of phosphorus fertilizers under the conditions of convection farming, which led to the adaptation of microorganisms to such conditions and their natural permanent application of large number of phosphorus fertilizers under convection agriculture, which led to the adaptation of microorganisms to such conditions and their natural selection on the basis of their relation to organic and inorganic phosphorus [19].

So, under the conditions of permanent application of phosphates, microorganisms that were able to synthesize organic and inorganic acids, polysaccharides, siderophores or to break down organic phosphorus-containing compounds enzymatically, synthesizing non-specific acid phosphatases, phytases, etc., gradually became the most represented group. It is known that the total quantity of synthesized compounds is very small in comparison with that of applied phosphorus fertilizers (to convert them into an available form).

A large number of microorganisms with high cellulolytic capacity were found in the soils of organic farming areas (Table 3). The greatest spectrum of such agronomically useful properties is demonstrated by isolates 3b, 11b, 16b and 3m, 5m, 10m.

For phosphate-solubilizing isolates, we did not find a clear difference in soils with different types of agriculture (like for cellulose), therefore, an experiment was carried out on the ability to transform it with selected isolates. Thus, for the second stage of the work, we selected isolates that were able to simultaneously transform organic and inorganic phosphates and also show cellulolytic activity. These were bacterial strains 3b, 11b from the soil under organic farming and 6b, 13b, 16b, 18b, 19b from the soil under convection farming, and strains of micromycetes 3m, 5m, 10m from soil under organic farming, and strain 8m from the soil under convection farming.

These isolates showed the highest activity on solid media, so their properties were tested in a liquid NBRIP medium, which contains insoluble calcium orthophosphate. After incubation of the isolates for 72 hr at 28 °C and 200 rpm, the content of dissolved phosphorus and the pH level in the liquid culture were determined (Fig. 2).

Fig. 2 indicates that the content of dissolved phosphorus in the culture increased by 5—7 times compared to the control. Micromycete 8 m from the soil of convection farming was the most active in orthophosphate mobilization. Micromycetes isolated from the soil of organic farming were characterized by a low ability to transform phosphate. At the same time, almost all of the selected bacterial isolates had approximately the same, rather high ability to dissolve this phosphate. It should be noted that the pH of the culture liquid of cultivated microorganisms changed from 7.0 to 5.1—5.3. It was shown that phosphorus solubilization also correlates with a decrease in the pH of the medium.

Micromycetes deserve special attention among phosphate-mobilizing microorganisms, because their content in the soil is about 0.1—0.5% of the total amount of microfungi [20, 21].

Moreover, phosphorus-solubilizing microscopic fungi do not lose the activity of dissolving phosphorus during prolonged cultivation in laboratory conditions, as in the case of phosphorus-solubilizing bacteria [22]. Therefore, they are the most promising for the creation of biological preparations in the agricultural industry. taking into account our data on the liquid NBRIP medium, isolate 8m is of particular interest and so it was selected for preliminary identification.

One of the important agronomically useful properties of microorganisms is their ability to antagonize phytopathogens. Selected isolates were tested for their ability to inhibit phytopathogenic microorganisms (Table 4).

First of all, it should be noted that all isolates capable of suppressing phytopathogens were from the chornozem of agricultural plots under organic farming. Isolates of bacteria 18b, 19b and isolate of micromycetes 8m demonstrated the absence of fungicidal action against the investigated phytopathogenic test strains *Botrytis cinerea, Fusarium oxysporum,* and *Xanthomonas phaseoli*. With using the dual plate culture, it was found that, due to its fungicidal effect, the largest growth inhibition zone was formed by bacteria 3b and micromycete 10m isolated from soil under organic farming.

The studies have shown that the isolates of micromycetes exhibited an inhibitory effect against phytopathogens, mainly due to the rapid uptake of the nutrient medium. As a result, they occupied a large area of the nutrient substrate, which blocked the growth of test cultures of phytopathogens. Often, upon direct contact of isolates with

Table 2. **Screening of isolates of bacteria and micromycetes obtained from the soil of agricultural areas under convection farming**

Isolate Number*	Ability to		
	mobilization of organic phosphates	mobilization of inorganic phosphates	cellulolytic activity
1b	+	+	—
6b	++	++	+
7b	+	+	-
8b	+	—	—
9b	+	+	—
13b	++	++	+
15b	+	+	+
18b	++	+	—
19b	++	+	—
1m	+	—	—
4m	+	+	—
8m	++	++	+
9m	+	+	—
11m	+	+	—

«+» — able to grow; «++» — able to form transparent zones around colonies, or to destroy paper; *b — bacteria; m — micromycetes.

Table 3. **Screening of isolates of bacteria and micromycetes obtained from the soil of agricultural areas under organic farming**

Isolate Number*	Ability to		
	mobilization of organic phosphates	mobilization of inorganic phosphates	cellulolytic activity
3b	++	+	—
4b	+	+	++
11b	++	+	—
16b	++	+	+
17b	+	—	—
20b	+	+	+
2m	+	+	+
3m	+	+	++
5m	+	+	++
6m	+	+	+
10m	+	+	++

«+» — able to grow; «++» — able to form transparent zones around colonies, or to destroy paper; *b — bacteria; m — micromycetes.

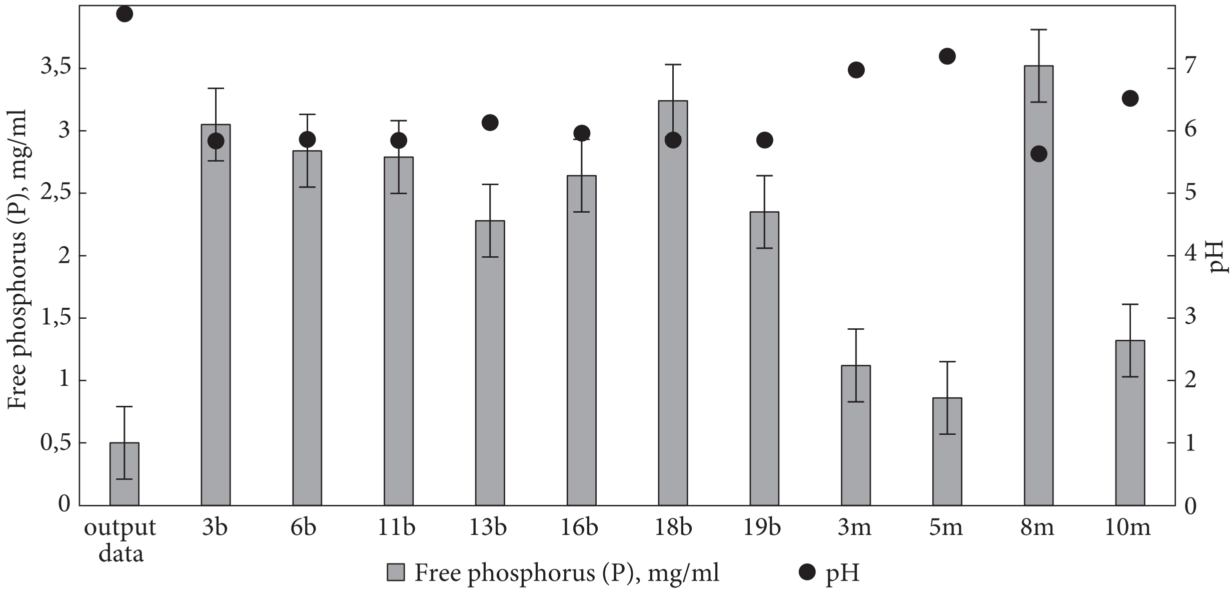

Fig. 2. Dissolved phosphorus concentration and pH after incubation of phosphate-mobilizing isolates

Table 4. **Antagonistic activity of selected isolates of bacteria and micromycetes against some phytopathogenic microorganisms**

Isolate number*	Diameter of zone inhibition of test culture, mm			Activity by the method of double counter cultures (+)		
	Botrytis cinerea	*Fusarium oxysporum*	*Xanthomonas phaseoli*	*Botrytis cinerea*	*Fusarium oxysporum*	*Xanthomonas phaseoli*
Isolates from soil under convection farming						
6b	—	—	—	—	—	+
13b	8.6	13.4	—	++	++	+
18b	—	—	—	—	—	—
19b	—	—	—	—	—	—
8m	—	—	—	+	+	+
Isolates from the soil under organic farming						
3b	19.8	24.2	5.1	++	++	++
11b	—	—	—	—	—	+
16b	—	—	—	—	—	+
3m	9.8	1.2	2.1	++	+	+
5m	—	3.2	—	+	++	+
10m	12.7	8.3	2.9	++	+	++

«—» — lack of phytopathogens suppression; «+» — phytopathogens suppression; «++» — phytopathogens suppression with the formation of a sterile zone; *b — bacteria; m — micromycetes.

the test strain, the pathogen mycelium was partially destroyed. Therefore, for the final stage of this work, these two isolates were selected, namely 3b from the soil of organic farming and 8m from the soil of convection farming, , and preliminary identification of the isolated agronomically useful microorganisms was carried out.

We performed Gram staining and microscopy of the bacterial isolate 3b and showed that it is a rod-shaped Gram-positive spore-forming bacteria. The physiological and biochemical properties of this strain testified that it is a fast-growing culture. On MPA within 1-3 days, it forms round, with slightly uneven margins, grayish matte colonies 2-14 mm in diameter, able to grow at a temperature of 10-45 °C and pH 4-9. According to biochemical tests (API KIT): catalase activity (+), oxidase activity (—), consumption of carbon sources: fructose (+), (D) + glucose (+), galactose (—), maltose (+), (D) + mannose (—), (D) + mannitol (+), (D) + xylose (—), ribose (+), glycerin (+), NO_3 reduction (variable), NO_2 reduction (variable), the ability to hydrolyze: casein (+), starch (+), gelatin (—), Voges-Proskauer(VP) test: pH <6 (variable) methyl red (MR) (—) test, indole test (—), use of citrate (+). According to biochemical and cultural data, isolate 3b was assigned to the *Bacillus sp.*, according to Bergey`s classification.

The carried out cultural-morphological and physiological-biochemical studies of micromycetes 8m have revealed the following features of the strain: on Czapek`s medium or PDA, it forms velvet colonies of pale to deep green color within 3—4 days; the reverse of the light green conidiophores looks like lateral branches of the surface mycelium, and round conidia (2—3 μm) are formed within 6—7 days and collected in spherical heads; chlamydospores can be formed (sometimes inside hyphalic cells) at 20 °C within 10 days [23]. Chlamydospores are usually unicellular and end in short hyphae. The culture grows at temperatures from 4 to 35 °C (optimal 24—28 °C) and pH 5—7.2 (optimal 6.0—6.5). It grows well on glucose, sucrose, and lactose. It has been shown that salts of organic acids (citric, oxalic) can inhibit the culture growth and development. It efficiently assimilates peptone, asparagine, sodium nitrate, hydrolyzed starch and fiber, and liquefied gelatin. Accordingly, based on the identification tests and culture parameters, the 8m isolate was assigned to the genus *Trichoderma,* family *Hypocreaceae*.

Discussion. Due to anthropogenic activities, in particular to the introduction of organic and mineral fertilizers, the environmental conditions in soils change significantly, leading to a significant adjustment in the taxonomic structure of microbial cenoses and their functional activities. Particularities of soil-climatic conditions also significantly affect species composition and features of the functioning of microbocenoses in soils. Therefore, when choosing the most effective methods of soil cultivation in each particular climate zone, a lot of attention should be paid to their biological assessment. Some studies have shown that fertilizer and lime have a positive effect on the prokaryote complex of soil microorganisms of ever-frozen soils [24].

Kremer and Means noted that herbicides reduce the total microbial population within 7 to 30 days (depending on the type of herbicidal molecules) after application [25]. They indirectly adversely affect microbial biodiversity by altering the physiology or biosynthetic mechanisms. This, in turn, affects the soil enzymatic activity, cellular membrane composition, protein biosynthesis, and the amount of plant growth regulators (gibberellins synthesis, transportation of indoleacetic acid , ethylene concentration, etc.). The application of excessive doses of herbicides leads to the death of many sensitive microorganisms [5].

The native soil microbiota is very important to maintain the environment quality , but because of the intensive use of agrochemicals, the microbial biomass changes, and the formation of large toxic waste is observed in the soil, groundwater, and surface water. According to PERMANOVA analysis, a greater effect of the herbicide persistence time in the soil and the effect of the herbicide class as well as the effect of interaction between these two factors are observed. In conclusion, the selective pressure exerted by the presence of these herbicides alters the composition of the local microbiota, atrazine and diuron, which most significantly affect the bacterial community in soil, and the herbicide 2,4-D was the one that less altered the microbial community and so the latter bacterial community was reestablished first [26]. Plant growth-promoting Streptomyces species were used as natural alternatives to synthetic fungicides. Using a high throughput sequencing method and microbiome profiling, the dynamics of rhizosphere bacterial communities manipulated by phosphate solubilizing Streptomyces strain was explored and correlated with higher plant growth promotion and disease suppression. In addition to enzyme activities, investigations on other characteristics of superior biocontrol strains of Streptomyces such as a secondary metabolite profile and their impact on the assembly of the rhizospheric bacterial communities' subsequent pathogen attack could be valuable to optimally design and develop SynComs of Actinobacteria for improving agricultural productivity and environmental sustainability [27].

The known effects of triazole fungicides on soil health are dose- and time-dependent. They may affect soil health either through direct application against soilborne fungal pathogens and also indirectly through spraying them on foliar surfaces. High doses of triazole fungicides greatly disturb the structure of the microbial communities in soil and usually lead to the diminution of the soil microbial population and a decrease in the activities of enzymes found in soil. This illustrates the importance of following the recommended dose for each type of crop and soil not to produce long-term effects on the soil properties and activity. The biostimulating substances are useful for reducing or neutralizing the adverse effect of these fungicides on soil microbial functions and biochemical processes. There is lack of information concerning the effects produced by metconazole, myclobutanil, and triadimenol on the soil microbiota and the effects of cyproconazole, epoxiconazole, flutriafol, metconazole, tetraconazole, triadimenol, and triticonazole on the activity of soil enzymes [28].

In our research, we have identified the regularities of the restructuring of soil microbial cenoses under convection and organic farming. Under the influence of intensive technologies, using a high content of pesticides, an increase in the number of oligonitrophilic and phosphate-solubilazing microorganisms occurs, mainly due to the inaccessibility or critical excess of certain microelements in these soils, and as a result stimulates the development of microorganisms capable of converting their insoluble forms into soluble ones. At the same time, there is observed a decrease in the number of microorganisms capable of assimilating mineral nitrogen, micromycetes, streptomycetes, and ammonificating bacteria.

Conclusions. The overwhelming number of bacteria capable of transforming phosphates was isolated from the soil of agricultural plots under convection farming, whereas isolates capable of dissolving cellulose were from the soil under organic farming. It was revealed that in the soils of convection farming, microorganisms capable of mobilizing exactly organic phosphates predominate. All isolates capable of significant inhibition of phytopathogens were from black soil (chornozem) of agricultural plots under organic farming.

Also, the primary identification of isolates was carried out, which turned out to be representatives of the genera *Bacillus* and *Trichoderma*. These isolates are important for further research with the prospect of creating a complex biological preparation with fungicidal properties and the ability to mobilize organic and inorganic phosphorus compounds.

REFERENCES

1. Kurdysh IK. Rol mikroorhanizmiv u vidtvorenni rodiuchosti gruntiv Silskohospodarska mikrobiolohiia. 2009; 9:7—32. Ukrainian. https://doi.org/10.35868/1997-3004.9.
2. Zvjagincev DG. Pochva i mikroorganizmy. Moskva: MGU; 1987. Russian.
3. Patyka VP, Symochko LIu. [Soil microbiological monitoring of natural and transformed ecosystems in the trans-Carpathian region of Ukraine]. Mikrobiol Z. 2013; 75(2):21—31. Ukrainian.
4. Bobryk NIu, Kryvtsova MV, Nikolaichuk VI, Voloshchuk IS. Reaktsiia mikrobioty gruntu na diiu vazhkykh metaliv u zoni vplyvu zaliznychnoho transportu. Visnyk Dnipropetrovskoho universytetu. Biolohiia, ekolohiia. 2016; 24(1):151—156. Ukrainian.
5. Meena RS, et al. Impact of agrochemicals on soil microbiota and management: Review. Land. 2020; 9(34):1—21. https://doi.org/10.3390/land9020034
6. Chi-Chu Loa. Effect of pesticides on soil microbial community. Journal of Environmental Science and Health. 2010; 45:348—359. https://doi.org/10.1080/10934520903467873.
7. Hussain S, Siddique T, Saleem M, Arshad M, Khalid A. Impact of pesticides on soil microbial diversity: enzymes and biochemical reactions. Advances in Agronomy. 2009; 102:159—200. https://doi.org/10.1016/S0065-2113(09)01005-0.
8. DSTU ISO 10381-1:2004 Yakist gruntu. Vidbyrannia prob. Chastyny 1—5. K.: Derzhspozhyvstandart Ukrainy. 2006. Ukrainian.
9. DSTU ISO 4287:2004 Yakist gruntu. Vidbyrannia prob. K.: Derzhspozhyvstandart Ukrainy. 2004. Ukrainian.
10. Volkogon VV, Nadkernichna OV, Tokmakova LM, ta in. Eksperymental'na gruntova mikrobiologiya: monografiya. Kyiv: Agrarna Nauka; 2010. Ukrainian.
11. Antypchuk AF, Piliashenko-Novokhatnyi AI, Yevdokymenko TM. Praktykum z mikrobiolohii. Vidkrytyi mizhnar. un-t rozv. liudyny «Ukraina». Kyiv: Un-t «Ukraina»; 2011. Ukrainian.
12. Humaira Y, Asghari B. Isolation and characterization of phosphate solubilizing bacteria from rhizosphere soil of weeds of khewra salt range and attock. Pakistan Journal of Botany. 2011; 3:1663—1668.
13. Khrystenko AO. Rozrobka standartu Ukrainy na metody vyznachennia rukhomykh spoluk fosforu y kaliiu u hruntakh. Visnyk ahrarnoi nauky. 2003; 6:9—13. Ukrainian.
14. Zhang J, Wang P, Fang L, Zhang Q-A, Yan C, Chen J. Isolation and Characterization of Phosphate-Solubilizing Bacteria from Mushroom Residues and their Effect on Tomato Plant Growth Promotion. Polish Journal of Microbiology. 2017; 66(1):57—65. https://doi.org/10.5604/17331331.1234993.
15. Brenner Don J, Krieg Noel R, Staley James T. The Proteobacteria. Bergey's Manual of Systematic Bacteriology 2nd Ed. New York: Springer, 2005. 1388 p.
16. Nassal D, Spohn M, Eltlbany N, et al. Effects of phosphorus-mobilizing bacteria on tomato growth and soil microbial activity. Plant and Soil. 2018; 427:17—37. https://doi.org/10.1007/s11104-017-3528-y.
17. Zavarzin GA, Kolotilova NN. Vvedenie v prirodovedcheskuju mikrobiologiju. Knizhnyj dom Universitet. 2001. Russian.
18. Tsyuk OA, Kyrylyuk VI, Yushchenko LP. Biochemical Activity of Typical Chernozem in Different Farming Systems. Mikrobiol Z. 2017; 79(3):65—71. https://doi.org/10.15407/microbiolj79.03.065.
19. Patyka NV, Tonkha OL, Patyka TI, Kiroyants MO, Veretyuk SV. Estimation of Prokaryotic Complex Methagenom of Chernozem Under Agricultural Use. Mikrobiol Z. 2018; 80(6):109—122. https://doi.org/10.15407/microbiolj80.06.109
20. Sharma SB, Sayyed RZ, Trivedi MH, Gobi TA. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soil Springer Plus. 2013; 2(587):1—14. https://doi.org/10.1186/2193-1801-2-587.
21. Bünemann EK, Oberson A, Frossard E. Solubilization of phosphorus by soil microorganisms. Phosphorus in action. Berlin: Springer; 2011.
22. Jacoby R, Peukert M, Succurro A, Koprivova A, Kopriva S. The Role of Soil Microorganisms in Plant Mineral Nutrition-Current Knowledge and Future Directions. Frontiers in plant science. 2017; 8(16):1—19. https://doi.org/10.3389/fpls.2017.01617.
23. Chaverri P, Samuels G. *Trichoderma/Hypocrea* (*Ascomycota*. *Hypocreales*. *Hypocreaceae*): species with green ascospores. Studies in Mycology. 2003; 48:100—116.

24. Tikhanovsky N. Change of microbiological properties of disturbed permafrost soils during their restoration under the influence of fertilizers. Ukrainian Journal of Ecology. 2020; 10(5): 36—43. https://doi.org/10.15421/2020_203.
25. Kremer RJ, Means NE. Glyphosate and glyphosate-resistant crop interactions with rhizosphere microorganisms. Eur J Agron. 2009; 31, 153—161. https://doi.org/10.3390/land9020034.
26. Moretto JAS, Altarugio LM, Andrade PA, Fachin AL, Andreote FD, Stehling EG. Changes in bacterial community after application of three different herbicides. FEMS Microbiology Letters. 2017; 364(13):1—6. https://doi.org/10.1093/femsle/fnx113.
27. Abbasi S, Spor A, Sadeghi A, Safaie N. Streptomyces strains modulate dynamics of soil bacterial communities and their efficacy in disease suppression caused by Phytophthora capsici. Scientific Reports. 2021; 11(1):11—16. https://doi.org/10.1038/s41598-021-88495-y
28. Roman DL, Voiculescu DI, Filip M, Ostafe V, Isvoran A. Effects of Triazole Fungicides on Soil Microbiota and on the Activities of Enzymes Found in Soil. A Review Agriculture 2021; 11:893—911. https://doi.org/10.3390/agriculture11090893

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ВЛАСТИВОСТІ МІКРООРГАНІЗМІВ, ВИДІЛЕНИХ ІЗ ҐРУНТІВ ПРИ ЗВИЧАЙНОМУ ТА ОРГАНІЧНОМУ ЗЕМЛЕРОБСТВІ

Незважаючи на значну увагу дослідників до різноманіття та функціонування мікробіоценозів ґрунту, досі недостатньо висвітлено питання щодо їх змін за умов інтенсивного землеробства. А питання щодо агрономічно корисних властивостей мікроорганізмів, які населяють ґрунти конвекційного землеробства, залишається взагалі не розкритим. У статті представлено результати досліджень, **метою** яких було визначити вплив різних способів обробітку ґрунту на функціональне різноманіття мікробіоти у ґрунті. Для дослідження використовували зразки ґрунтів із сільськогосподарських ділянок Київської області, які містять рослинні залишки та зазнали впливу конвекційного та органічного землеробства. Методи. Органічне землеробство означало мінімальну обробку ґрунту та повну відсутність внесення будь-яких пестицидів протягом останніх п'яти років. Аналіз мікробіоти ґрунту проводили з використанням диференційно-діагностичних живильних середовищ методом серійних розведень ґрунтової суспензії. Для кількісної оцінки фосфатмобілізувальних властивостей виділених мікроорганізмів проводили вимірювання концентрації фосфору в розчині (вирощували в рідкому середовищі NBRIP) та виявляли спектрофотометричним методом Ареніуса на спектрофотометрі Ulab 102UV Spectrophotometer. Попередню ідентифікацію відібраних ізолятів здійснювали за допомогою експрес-систем ідентифікації бактерій API KIT® (bioMérieux, Франція). Відібрані ізоляти засівали в комерційні набори API ID32C, API 50CHB та інкубували впродовж 48 годин при 28 ± 2 °C. Отримані результати порівнювали з профілями інтернет-бази даних відповідного набору (API database). Статистичний аналіз та визначення кореляційних залежностей одержаних результатів проводили за допомогою стандартних комп'ютерних програм Statistica 8.0 та Microsoft Excel 2010. **Результати**. Для проведення порівняльного дослідження щодо кількості агрономічно корисних мікроорганізмів у досліджуваних ґрунтах було відібрано ізоляти. Вони характеризувалися тинкторіальними властивостями, притаманними мікроорганізмам, здатним до мобілізації органічних та неорганічних форм фосфатів або до деструкції целюлози. У загальній кількості було виділено 24 ізоляти типових ґрунтових бактерій та 12 ізолятів мікроміцетів. Ґрунт ділянок, де застосовували органічну агротехнологію вирощування, відзначався вищою чисельністю мікроорганізмів усіх еколого-трофічних груп, за винятком олігонітрофільних та фосфатмобілізувальних. Переважна більшість бактерій, здатних до трансформації фосфатів, була виділена з ґрунту сільськогосподарських ділянок, які зазнали впливу конвекційного землеробства. Найбільша кількість ізолятів, здатних до розкладу целюлози, виділена з ґрунту ділянок органічного землеробства. Найширшим спектром агрономічно корисних властивостей, зокрема, здатністю до мобілізації органічних та неорганічних фосфатів та целюлозолітичною активністю, володіють 5 ізолятів: 6b, 13b, 18b, 19b та 8m. Після інкубації ізолятів на середовищі NBRIP протягом 72 годин при 28 °C та 200 об/хв проведено аналізи на вміст розчиненого фосфору та рівня pH в культуральній рідині. Найбільшою фосфатмобілізувальною активністю характеризувався ізолят 8m, виділений із чорноземного ґрунту конвекційного землеробства та класифікований нами як *Trichoderma* sp. Виявлено, що переважну більшість бактерій, здатних до трансформації фосфатів було виділено з ґрунту сільськогосподарських ділянок, які зазнали впливу конвекційного землеробства, а ізоляти, здатні до розкладу целюлози, — з ґрунту ділянок органічного землеробства. Всі ізоляти, здатні до суттєвого пригнічення фітопатогенів були виділені з чорнозему сільськогосподарських ділянок органічного землеробства. **Висновки**. Первинна ідентифікація ізолятів дозволила віднести їх до родів *Bacillus* та *Trichoderma.* Ці ізоляти є важливими для подальших досліджень із перспективою створення комплексного біологічного препарату з фунгіцидними властивостями та здатністю до мобілізації органічних і неорганічних сполук фосфору.

Ключові слова: *фосфатмобілізувальні мікроорганізми, агрономічно цінні мікроорганізми, целюлозолітичні мікроорганізми, чорнозем.*