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Screening of Microorganisms with High Biological Activity to Create Consortia as a Growth Stimulator for Wheat Seeds

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ABSTRACT

Addressing the pressing need for more sustainable farming practices that concurrently enhance crop productivity, this study focuses on the identification of beneficial microorganisms and their impact on wheat seed germination. Through rigorous screening of microorganisms hailing from the wheat rhizosphere, a targeted approach was adopted to formulate microbial consortia, aiming for an additive effect in boosting plant growth. In the initial stage, a comprehensive screening was conducted on microorganisms isolated from the wheat rhizosphere soil. Subsequently, the influence of the culture liquids from these isolates, along with those of selected microorganism strains from established collections, on the growth rates of wheat was meticulously examined. These methodical investigations were instrumental in the formation of the microbial consortia. From an extensive pool of 35 collection strains and 16 isolates, microorganisms demonstrating the most significant positive impact on wheat growth were selectively chosen. Three potent consortia were subsequently formulated from these beneficial microorganisms. Although these findings are yet to be validated through practical application, the results offer promising prospects for their utilization in the agricultural sector. The identified microbial consortia present a green alternative to conventional fertilisers, thereby potentially contributing to the advancement of sustainable agriculture practices.

1. INTRODUCTION

The term "high biological activity" refers to the presence and effectiveness of biological agents, such as enzymes, hormones, and other compounds, in a crop or plant [1]. These agents can have a significant impact on the plant's growth and development, as well as on its overall health and ability to resist diseases and pests. The criteria for determining whether a crop has high, low, or medium biological activity can vary depending on the specific plant species, the environment in which it is grown, and other factors. However, some general guidelines that can be used to assess biological activity in crops include: yield, quality, nutrient content, and metabolism.

The alternative to increasing the area for crops is to increase crop productivity. Various fertilisers are used to increase the biomass: sources of nitrogen, phosphorus, potassium. At the same time, the use of endogenous substances has its drawbacks. Thus, when urea and ammonium nitrate are introduced as a source of nitrogen, losses of these substances are observed as a result of redox processes in the soil and, as a consequence, incomplete assimilation of nitrogen by plants. In addition, the loss of nitrogen gas in the form of oxides and the leaching of nitrates from the soil also introduces environmental risks of using conventional nitrogen fertilisers. One of the environmentally safe ways to stimulate plant germination and increase biomass is the use of biofertilisers and biologics. The key advantage of biological products is their natural origin - these are isolated and selected microorganisms that improve the natural processes of fixation and assimilation of biogenic components by plants. Such biological products are produced based on isolated soil microorganisms and do not have a cumulative effect on the soil and toxic or carcinogenic effects on humans. The use of biological products has several areas. For example, biological products can have a protective effect on plants: protect against fungal or bacterial infections. In addition, an integral task of the development of biological products is to increase the germination and growth of plant biomass. It is believed that one of the mechanisms of the positive effect of biological products on germination and plant growth are phytohormones produced by a consortium of microorganisms [2].

After studying the role of rhizosphere microorganisms on radish plants, in 1978, Bhattacharyya and Jha [3] introduced the term "rhizobacteria", which they described as a population of microorganisms inhabiting the rhizosphere zone and positively influencing plant growth and development. After further study of the role of rhizobacteria in these populations, the term "plant growth-promoting rhizobacteria" (PGPR) - rhizobacteria that stimulate plant growth - was introduced. There are several ways for rhizobacteria to interact with the host organism: neutral, negative, and positive effects. For example, most microorganisms inhabiting the root zone of plants are commensals and have no effect on the growth and development of the host organism. In contrast to commensalism, there are rhizobacteria that negatively affect the growth of the host plant. Such microorganisms secrete

phytotoxic substances, such as ethylene, hydrogen cyanide, which inhibits the growth and development of plants. The third group of rhizobacteria has a positive effect on the host body. Thus, rhizobacteria of this group contribute to the assimilation of nitrogen and other nutrients by plants, secrete stimulating phytohormones, and can have the effect of protecting plants from parasitic microorganisms in a competitive way. In addition, rhizobacteria of this group have the properties of neutralising toxic substances for the plant and a stimulating effect on the development of the rhizosphere. Among the endophytic bacteria that promote nitrogen uptake by plants, there are such genera of bacteria as Agrobacterium, Arthrobacter, Azotobacter, Azospirillum, Bacillus, Burkholderia, Caulobacter, Chromobacterium, Erwinia, Flavobacterium, Micrococcus, Pseudomonas, and Serratia. In turn, actinomycetes are also important, as their colonisation of the rhizosphere helps to protect plants against a number of pathogenic fungi. Certain types of streptomyces contribute to the protection of plants from fusariosis in a competitive way [4].

To use rhizobacteria as biological products, the necessary stage is the isolation of microorganisms from the rhizosphere of the soil, screening for biological activity, and selection of the most effective bacteria. That is why it is relevant to screen and study microorganisms that have a positive effect on the growth and protection of plants from pests. Selection and development of effective biological products is carried out through screening of microorganisms and further investigation of the effect of certain bacterial strains and their combinations [5]. The development of the field of eco-friendly agriculture, and the search for new ways to increase crop yields, are the reason for the study of new strains and the creation of consortia of microorganisms to increase plant yields in an environmentally safe way.

The purpose of the study was to isolate and select cultures of microorganisms for the further formation of consortia and increase the germination of seeds, and growth indicators of wheat plants. To achieve this goal, collectable strains of microorganisms and isolates from the root zone of wheat were used. Isolates and collection strains were studied for their biological activity in relation to the processes of wheat plant growth and the creation of the most favourable consortia.

2. MATERIALS AND METHODS

For the isolation of microorganisms from the rhizosphere of wheat soils, a 1 g sample was taken and 100 ml of sterile tap water was added. For further use of the soil suspension, it was diluted in a ratio of 1:100. The suspension was seeded on dense nutrient media in dilutions of 1:10, 1:100, and 1:1000. The choice of the multiplicity of breeding was substantiated by the type of bacterial culture and the properties of the soil. Under sterile conditions, 1 ml of soil suspension was applied to the solid media and spread evenly over the surface with a spatula. Petri dishes were placed in a thermostat and cultivated for a period of time determined by the type of medium and microorganism's characteristic of it. Thus, for plain agar, accounting was carried out after 2-3 days for the presence of spore and non-spore forms of bacteria. For Czapek and Gause agar medium, colonies of fungi and actinomycetes were counted on days 5-7 of growth; for Sabouraud agar medium, yeast colonies were counted on days 2-3 of growth [6, 7]. To identify isolates isolated from the soil rhizosphere, a study of the cultural and morphological-physiological parameters of microorganisms was carried out in accordance with Bergey's manual [8].

The growth-stimulating effect of collection strains and isolates was studied by growth indicators and the percentage of germination of wheat seeds. Wheat seeds were treated with cultures of microorganisms previously grown on a liquid nutrient medium of meat-infusion broth. Microorganisms were grown on Innova44-R shaker (United States of America (USA), 2008) at a temperature of 35-37°C for 1-2 days. The culture liquid (CL) of the strain cells was diluted to concentrations: 1:10, 1:50, and 1:100. Sterile tap water was used as a control. 30 large wheat seeds were selected to investigate the effect of microbial metabolites on the growth and germination of seeds. The selected seeds were treated by the humidification method for 10 days. The estimated parameters were the percentage of germination of seeds, length and weight of sprouts [9, 10]. Consortia have been developed to select the most biologically active variants of microorganisms. For the development of the consortium, data on the effect of each of the investigated isolates and collection strains on growth indicators and seed germination efficiency were used. Thus, 3 consortia were formed, the data about the consortia are presented in Table 1.

Table 1. Variants of growth-stimulating consortia

Variants of Consortia	Bacterial Strains	Control
Consortium 1	*Bacillus pumilis Pol P3(1) 10 RKM0528, Bacillus thuringiensis Pb 30 RKM0341, Bacillus licheniformis 356 RKM0074,* isolate *Serratiamarcescens Sh-2*	Sterile tap water
Consortium 2	*Rhizobium legiminosarum B-6 RKM 0272, Azotobacterchrococcum Azp24 B-RKM 820, Bacillus pumilis Pol P3(1) 10 RKM0528,* isolate *Serratiamarcescens Sh-1*	Sterile tap water
Consortium 3	*Rhyzobiumleguminosarum RKM 0501, Azot. chrococcum C9 B-RKM 783, Pseudomonas alcaligenes H-15 RKM 0160, Enterobacter cloacae KB-2* isolate	Sterile tap water

For the developed consortia, the biocompatibility of bacterial strains was investigated by the method of joint cultivation by Glushanova and Blinov [11]. Environmental factors such as temperature, humidity, light, pH, and availability of nutrients were taken into account during the cultivation of the microbial colony. These factors are critical for cultivation and significantly affect the rate of growth, reproduction, and metabolic activity of microorganisms. A drop of culture medium containing cells of the culture was applied to the surface of the plain agar medium, after drying of which a drop of another culture was applied so that it covered part of the first one. Drops of the same culture applied to each other were used as a control. Thus, the biocompatibility of the cultures included in the consortium was investigated. The evaluation of the viability index of microorganisms included in the consortia was investigated by the Miles et al. [12] method. Statistical processing of the results was carried out using Statistica 6.0, Microsoft Excel 97 software suites. To analyse the data obtained, the parameters of the mean and standard deviation for the group of studied plants were used [13].

Table 2. Cultural and morphological characteristics of colonies and results of Gram staining of microorganisms isolated from wheat rhizosphere soils

Name of the Isolate	Cultural and Morphological Characteristics	Gram Staining
K-1	Colonies are brown, slightly convex, matte, the edges are smooth, the consistency is soft, diameter is 0.5-1.5 mm.	Rods, gram -
KB-1	Colonies are white, convex, shiny, the edges are smooth, the consistency is soft, diameter is 0.5-1 mm.	Cocci, gram +
KJ-1	Colonies are yellow, shiny, convex, smooth edges, diameter is 0.5-1 mm.	Rods, gram +
Sh-1	Colonies are brown, shiny, convex, the edges are smooth, the consistency is stringy, diameter is 0.5-1 mm.	Rods, gram -
TB-1	Colonies are white, flat, matte, smooth edges, diameter is 0.5-1 mm.	Cocci, gram +
ChK-4	Colonies are brown, convex, shiny, the edges are smooth, the consistency is soft, diameter is 1-2 mm.	Rods, gram +
K-2	Colonies are brown, convex, shiny, the edges are smooth, the consistency is soft, diameter is 0.5-1.5 mm.	Rods, gram +
KB-2	Colonies are white, convex, shiny, the edges are smooth, the consistency is soft, diameter is 0.5-1.5 mm.	Rods, gram +
Sh-2	Colonies are pinkish white, convex, shiny, smooth edges, soft consistency, diameter is 0.5-1.5 mm.	Rods, gram +
ShB-2	Colonies are milky, convex, shiny, the edges are smooth, the consistency is soft, diameter is 0.5-1.5 mm.	Rods, gram +
TB-2	Colonies are white, slightly convex, shiny, the edges are smooth, the consistency is soft, diameter is 0.5-1.5 mm.	Rods, gram +
TK-2	Colonies are light brown, convex, shiny, the edges are smooth, the consistency is soft, diameter is 0.5-1.5 mm.	Rods, gram +
K-3	Colonies are of light milky colour, convex, shiny, small, the edges are smooth, the consistency is soft, diameter is 0.5-1.5 mm.	Rods, gram -
Sh-3	Colonies are of light milky colour, convex, shiny, small, the edges are smooth, the consistency is soft, diameter is 0.5-1.5 mm.	Rods, gram -
T-3	Colonies are white, convex, the edges are rough, matte, the consistency is stringy, diameter is 0.5-1.5 mm.	Rods, gram +
AK-4	Colonies are white, flat, matte, dull, rough edges, soft consistency, diameter is 0.5-1.5 mm.	Rods, gram +

Table 3. Cultural and morphological characteristics of collection strains

Name of Collection Strains	Cultural and Morphological Features	Gram Staining
Bacillus licheniformis 356 RKM 0074	Colonies are milky, shiny, convex, the edges are smooth, the consistency is stringy.	Rods, gram+
Bacillus subtilis IKIRKM 0102	Colonies are milky, matte, flat, the edges are hairy, the consistency is stringy.	Rods, gram+
Bacillus thuringiensisA1RKM 0198	Colonies are milky, shiny, convex, the edges are smooth, the consistency is stringy.	Rods, gram+
Bacillus subtilis ZbRKM 0285	Colonies are milky, shiny, convex, the edges are smooth, the consistency is stringy.	Rods, gram+
Bacillus aguimarisAE1RKM0294	Colonies are milky, shiny, convex, the edges are smooth, the consistency is stringy.	Rods, gram+
Bacillus thuringiensis Pb30RKM 0341	Colonies are milky, shiny, convex, the edges are smooth, the consistency is stringy.	Rods, gram+
Bacillus cereus T1RKM 0438	Colonies are milky, shiny, convex, the edges are smooth, the consistency is stringy.	Rods, gram+
Bacillus brevis 35DMGRKM 0453	Colonies are light milky, shiny, convex, the edges are smooth, the consistency is viscous.	Rods, gram+
Bacillus megaterium F4RKM 0513	Colonies are milky, shiny, convex, the edges are smooth, the consistency is stringy.	Rods, gram+
Bacillus pumilisPolP3(1) 10 RKM 0528	Colonies are milky, shiny, convex, the edges are smooth, the consistency is stringy.	Rods, gram+
Bacillus subtilis G2RKM 0561	Colonies are white, dull, flat, the edges are wrinkled, the consistency is stringy.	Rods, gram+
Rhizobium legumiasarium ZGRKM 0193	Colonies are milky, matte, convex, the edges are even.	Cocci, gram+
RhizobiumcicerZNRKM 0194	Colonies are milky, flat, shiny, the edges are wrinkled, the consistency is stringy.	Cocci, gram+
Rhizobium leguminosarum B-6RKM 0272	Colonies are brown, flat, shiny, the edges are smooth, the consistency is stringy.	Rods, gram+
Rhizobium leguminosarum B-23RKM 0273	Colonies are light brown, flat, shiny, the edges are even, the consistency is stringy.	Rods, gram+
Rhizobium leguminosarum RKM 0501	Colonies are brown, convex, shiny, the edges are smooth, the consistency is stringy.	Rods, gram+
Rhizobium pusense Z-5RKM 0517	Colonies are milky, matte, flat, and the edges are wrinkled.	Cocci, gram+
Pseudomonas putida VG-84RKM 0060	Colonies are grey, translucent, the surface is rough, the edges are slightly wavy, the consistency is soft.	Rods, gram-
Pseudomonas alcaligenes H-15RKM 0160	Colonies are brown, shiny, edges are smooth, convex, the consistency is soft	Rods, gram-
Pseudomonas fluorescens A-3RKM 0196	Colonies are brown, translucent, round in shape, flat, smooth with even edges, the consistency is viscous.	Rods, gram-
Pseudomonas aeruginosa 36KRKM 0255	Colonies are brown, shiny, the edges are smooth, the consistency is soft.	Rods, gram-
Pseudomonas aeruginosa Zb32RKM 0284	Colonies are light brown, shiny, the edges are smooth, flat, the consistency is soft	Rods, gram-
Pseudomonas aeruginosa G23RKM 0286	Colonies are brown, shiny, the edges are even, flat, smooth, the consistency is soft.	Rods, gram-
Pseudomonas aeruginosa G13 RKM 0417	Colonies are brown, shiny, the edges are even, flat, smooth, the consistency is soft.	Rods, gram-

Agrobakteriumtumejacienes Z-2 B-RKM 0518	Colonies are light brown, shiny, convex, the edges are smooth, the consistency is stringy.	Rods, gram-
Agrobakteriumtumejacienes Z-9 B-RKM 0520	Colonies are light brown, shiny, flat, edged, the consistency is soft.	Cocci, gram+
Agrobakteriumrhizogenes M 4 B-RKM 0613	Colonies are light brown, shiny, flat, the edges are even, the consistency is viscous.	Cocci, gram+
Agrobakteriumtumejacienes AC-1B-RKM 0791	Colonies are milky, shiny, convex, the edges are smooth, the consistency is stringy.	Cocci, gram+
Azotobacterchroococcum Azal 2 B-RKM 0687	Colonies are milky, shiny, flat, rough edges, the consistency is stringy.	Rods, gram+
Azotobacterchroococcum Azal 1 B-RKM 0688	Colonies are milky, dull, flat, the edges are smooth, the consistency is stringy.	Rods, gram+
AzotobacterchroococcumC9 B-RKM 0783	Colonies are brown, dull, flat, the edges are rough, the consistency is stringy.	Rods, gram+
Azotobacterchroococcum Azp24 B-RKM 0820	Colonies are light brown, shiny, flat, the edges are even, the consistency is stringy.	Cocci, gram+
Streptomyces Collinus New 10RKM 615	Colonies are white, dense, pasty, folded.	Gram+, branched and aerial gifs
Streptomyces flavofungini RKM 717	Colonies are pinkish white, dull, dense, the consistency is pasty.	Gram+, branched and aerial gifs
Streptomyces ambofaciens 40 RKM 736	Colonies are white, dense, folded, the consistency is pasty.	Gram+, branched and aerial gifs

3. RESULTS AND DISCUSSION

3.1 Identification of bacterial isolates

After isolates were obtained from the rhizosphere of the wheat soil of the Akmola region, a study of the cultural and morphological characteristics of the isolated microorganisms was carried out. According to the results of such a study, it was found that microorganisms with different cultural and morphological characteristics were isolated from the soil. Thus, out of 16 isolated isolates, after Gram staining, 10 isolates with gram-positive rods, 4 isolates with gram-negative rods, 2 strains of coccoid microorganisms were identified. The data is presented in Table 2.

The cultural and morphological features of the collection strains were also investigated. Of 35 cultures, 7 were represented by gram-positive cocci (mono- and diplococci), 17 cultures - gram-positive rods, 8 strains - gram-negative rods. In addition, among the collection strains there were 3 strains with filamentous fungi (Table 3).

Identification of 16 bacterial isolates was carried out using the MALDI-Tof method of mass spectrometry [14]. The results of isolates identification are presented in Table 4.

Table 4. Results of isolates identification

No.	Isolates	Maldi-Tofmassspectrometr
1	*Sh-1*	*Delftiaacidovorans*
2	*TB-1*	*Bacillus cereus*
3	*KB-2*	*Enterobacter cloacae*
4	*Sh-2*	*Serratiamarcescens*
5	*TB-2*	*Enterobacter ludwiqii*
6	*K-1*	*Delftiaacidovorans*
7	*KB-1*	*Pseudomonas qessardii*
8	*KJ-1*	*Stenotrophomonasmaltophilia*
9	*K-2*	*Enterobacter ludwiqii*
10	*ShB-2*	*Enterobacter cloacae*
11	*TK-2*	*Serratiamarcescens*
12	*K-3*	*Enterobacter cloacae*
13	*Sh-3*	*Enterobacter cobei*
14	*T-3*	*Enterobacter cloacae*
15	*ChK-4*	*Staphylococcus epidermidis*
16	*AK-4*	*Pseudomonas fluorescens*

Thus, all isolates were identified as: *Sh-1 - Delftiaacidovorans, TB-1 - Bacillus cereus, KB-2 - Enterobacter cloacae, Sh-2 -Serratiamarcescens, TB-2 - Enterobacter ludwiqii, K-1 -Delftiaacidovorans, KB-1 - Pseudomonas qessardii, KJ-1 -Stenotrophomonas maltophilia, K-2 - Enterobacter ludwiqii, ShB-2 - Enterobacter cloacae, TK-2 - Serratiamarcescens, K-3 - Enterobacter cloacae, Sh-3 - Enterobacter cobei, T-3 - Enterobacter cloacae,ChK-4 - Staphylococcus epidermidis, AK-4 - Pseudomonas fluorescens.*

3.2 Results of the effect of microbial metabolites on seed germination and growth indicators of wheat plants

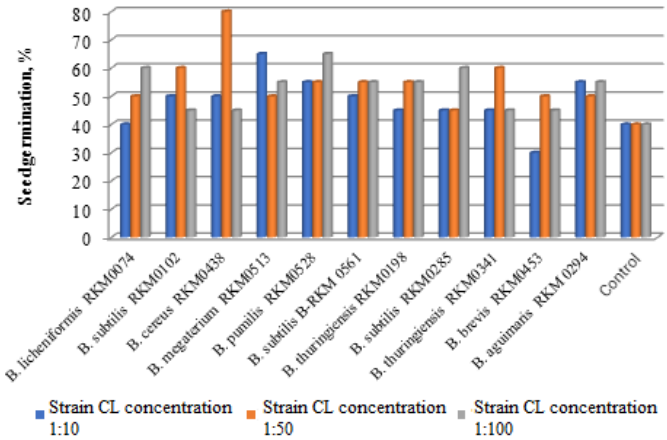

Figure 1. The effect of culture liquid of microorganisms of the genus Bacillus on the germination of wheat seeds

To investigate the effect of microbial metabolites on seed germination and growth indicators of wheat plants, a series of experimental studies were conducted, as a result of which strains and isolates with the greatest growth-stimulating activity were identified. Thus, for collection strains of the genus Bacillus, B. cereus microorganisms showed the greatest effect on the percentage of germination of seeds at a dilution of 1:50, while B. pumilis microorganisms showed the greatest efficiency at all three dilutions. So, the percentage of germination of wheat seeds was 55.55 and 65% for dilutions of 1:10, 1:50, and 1:100, respectively, when compared with

the control of 40% for all dilutions. The data is shown in Figure 1.

When comparing the effect on the growth indicators of wheat plants, B. cereus microorganisms had the greatest effect at 1:100 dilution: 12.5 compared to 8.2 for control. At the same time, for other dilutions, the effect of metabolites of the same strain was equal to the control (at 1:50) or less than the control (at 1:10) (Figure 2). Thus, although a growth-stimulating effect was observed during the treatment of seeds and plants with B. cereus culture liquid, an increase in the concentration of metabolites of this microorganism inhibited the growth and development of wheat plants. Among the strains of microorganisms of the genus Bacillus, the highest indicators for seed germination and growth activity were shown by B. pumilis microorganisms, with the sprout length of 9.4 mm, 8.8 mm, 11.6 mm for CL dilutions of 1:10, 1:50, and 1:100, respectively, with a control of 8.2 mm (Figure 3). The data is shown in Figure 2.

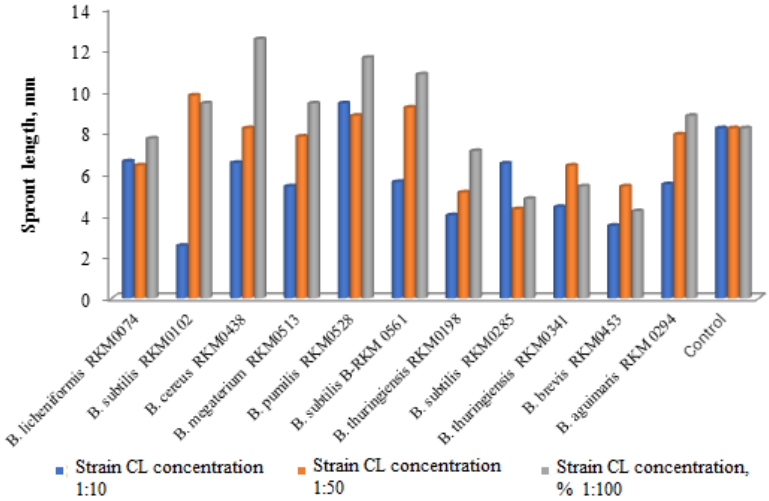

Figure 2. The effect of the culture liquid of microorganisms of the genus Bacillus on the sprout length of wheat plants

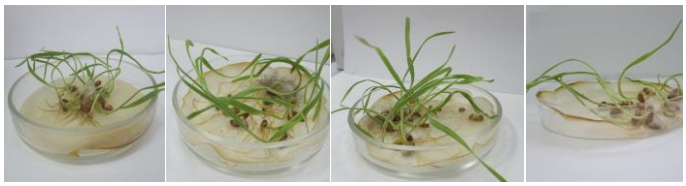

Figure 3. Treatment of wheat seeds with culture liquid B. cumilis PolP3(1) 10 RKM 0528 (on the 10th day)
Note: *a - CL 1:10; b - CL 1:50; c - CL 1:100; d - Control.*

Based on the results obtained, one of the most effective microorganisms as a biological stimulator of plant germination and growth is the B. pumilis. This microorganism is also characterised by the feature of inhibiting the growth of fungi of the Rhizoctonia and Fusarium families - pathogens of infectious plant diseases [15]. One of the mechanisms of influence on fungal organisms is the cleavage of chitin of the cell wall by enzymes of the microorganism B. pumilis. Other mechanisms of interaction of B. pumilis are being actively studied, which is complicated by the multifactorial effect of the interaction of bacterial and fungal cells. In addition, the B. pumilis microorganism is characterised by the property of plant protection from the influence of heavy metals, drought, and other abiotic stresses [16]. One of the mechanisms of plant protection from drought is considered to be an increase in the concentration of free amino acids, proteins, carbohydrates, conditioned by the influence of primary metabolites of the microorganism [17]. Such a feature of these microorganisms is a promising property for further use of this strain already as part of consortia. When studying the effect of metabolites of microorganisms of Rhizobium strains, it was determined that the greatest effect on seed germination rate was produced by the microorganisms Rhiz. cicer RKM 0194, Rhiz. legiminos. RKM 0272, Rhiz. legiminos. RKM 0273, Rhyz. leguminos. RKM 0501 and Rhiz. pusense RKM 0517. The treatment of wheat seeds with metabolites of these microorganisms resulted in the highest seed germination rate compared to the control. At the same time, the highest seed germination rates were for Rhiz. pusense RKM0517-75% seed germination at a dilution of 1:100, with a control indicator of 40%. The same high germination result was characteristic of Rhiz. pusense RKM 0517 and at a dilution of 1:50, which is the result of high biological activity of metabolites of cells of this strain. The results are presented in Figure 4.

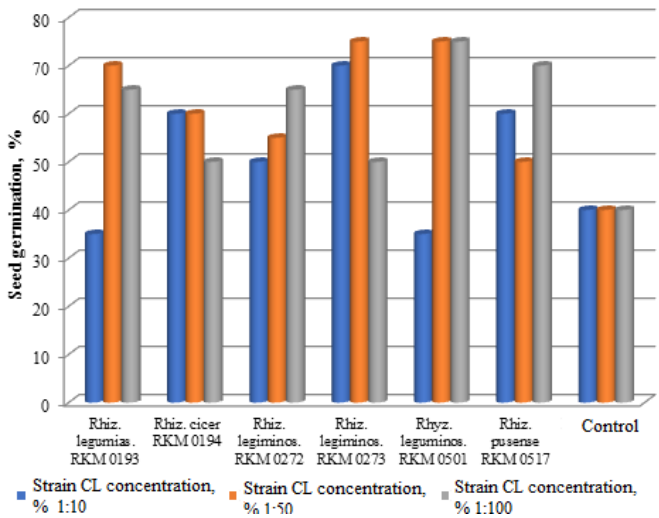

Figure 4. Effect of the culture liquid of Rhizobium microorganisms on the germination of wheat seeds

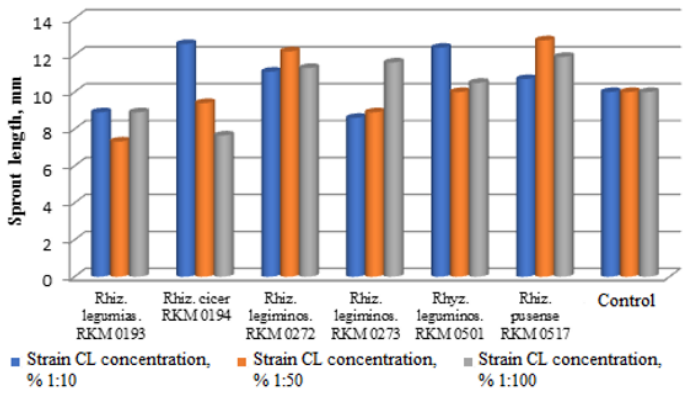

Figure 5. Effect of the culture liquid of microorganisms of the genus Rhizobium on the length of the sprout of wheat plants

When studying the effect of metabolites of microorganisms of the genus Rhizobium on the growth indicators of wheat plants, it was found that the greatest positive effect on plant growth is produced by metabolites of Rhiz. legiminos. RKM 0272, Rhyz. leguminos. RKM 0501 and Rhiz.pusenseRKM 0517. For these microorganisms, a positive effect was observed at all three dilutions of 1:10, 1:50, and 1:100. The data is shown in Figure 5. When studying the mechanisms of the impact of microorganisms of the genus Rhizobium on the development and growth of plants, it was shown that a positive effect of microorganisms is possible through the synthesis of siderophores, the ability to dissolve phosphate compounds in the soil and the ability of some species of Rhizobium spp. to nitrogen fixation [18]. At the same time, siderophores as iron chelating substances can be used not only to increase the efficiency of plant growth, but also in medicine and biotechnology.

For wheat seeds treated with culture liquid of microorganisms of the genera Agrobacterium, Azotobacter, Streptomyces, it was found that the greatest positive effect on the percentage of germination of wheat seeds was characteristic of strains of A. chrococc. B-RKM 783 and A.hizogenes RKM 0613. The data is shown in Figure 6.

When studying the effect of metabolites of microorganisms of the genera Agrobacterium, Azotobacter, Streptomyces, it was found that the most significant effect on growth indicators is carried by microorganisms A. chrococc. B-RKM 783, A. chrococc. B-RKM 820. At the same time for A. chrococc. B-RKM 820, values for the sprout length are observed greater than the control for all dilutions (1:10, 1:50, and 1:100). The data is shown in Figure 7.

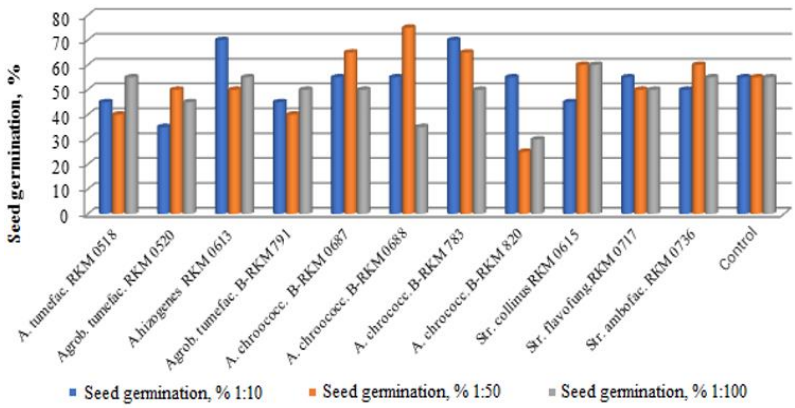

Figure 6. Influence of culture liquid of microorganisms of the genera agrobacterium, azotobacter, streptomyces on germination of wheat seeds

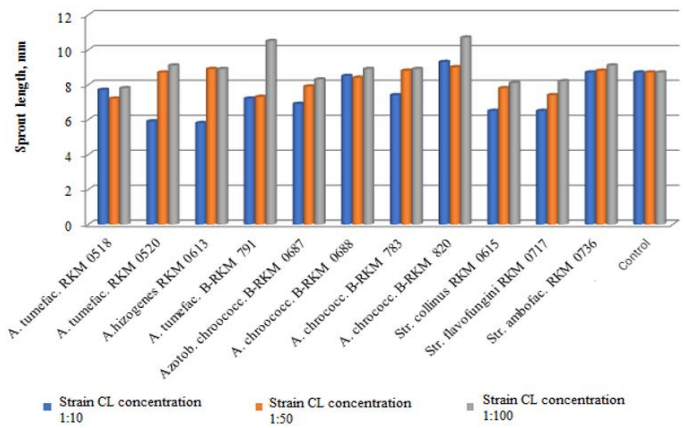

Figure 7. Effect of the culture liquid of microorganisms of the genera agrobacterium, azotobacter, streptomyces on the length of the sprout of wheat plants

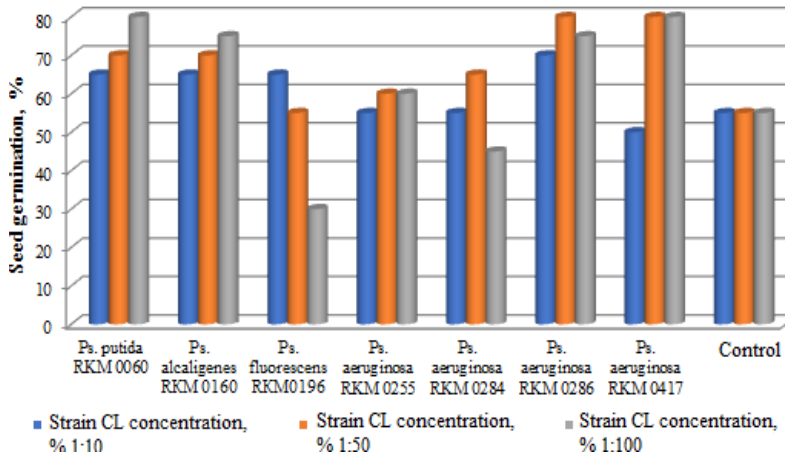

Figure 8. Effect of metabolites of microorganisms of the genus pseudomonas on the germination of wheat seeds

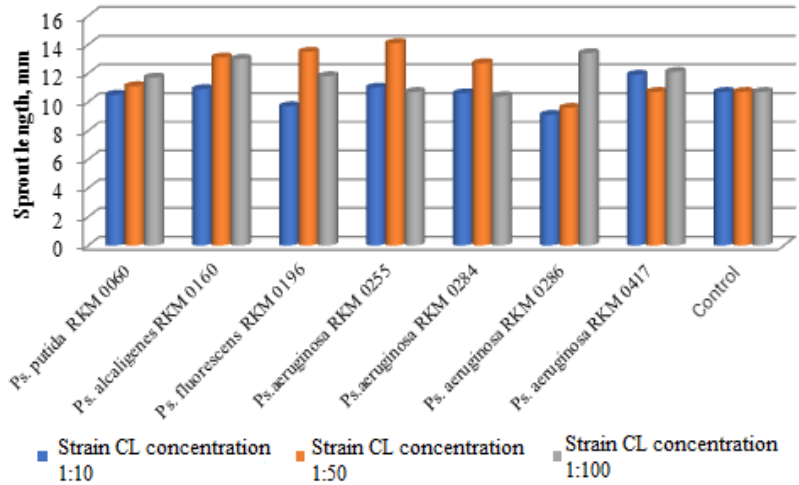

Figure 9. Influence of culture liquid of pseudomonas microorganisms on the length of wheat plant sprout

When studying the effect of metabolites obtained from the culture liquid of microorganisms of the genus Pseudomonas, it was found that the greatest positive effect on the germination of wheat seeds was observed when seeds were treated with metabolites obtained from the culture liquid of Ps. rutida RKM 0060, Ps. alcaligenes RKM 0160, Ps. aegidiposa RKM 0286 and Ps. aegidiposa RKM 0417. At the same time, for Ps. aegidinosa RKM 0417 culture, a positive effect was observed at dilutions of 1:50 and 1:100, whereas inhibition of the germination process of wheat seeds was observed when diluting the culture liquid at 1:10. The data is shown in Figure 8 and Figure 9.

An important stage of the screening of microorganisms and the creation of consortia was the investigation of the effect of metabolites of microorganisms-isolates selected from the rhizosphere of wheat soil. Thus, out of 16 selected isolates, the greatest positive effect on the percentage of germinated seeds was shown by Ser. Marcescens Sh-2, Delft. acidovorans Sh-1, Stenotroph. Maltophilia CL-1, B. cereus TB-1, Ent. Cloacae KB-2. At the same time, for an isolate identified as Ent. cloacae KB-2, percent germination of wheat seeds at 1:10, 1:50, 1:100 was 90%, 85% and 90%, respectively, which exceeds the control indicators of 80%. The data is presented in Figure 10.

Isolates of microorganisms Ser. marcescens Sh-2, Delft. acidovorans Sh-1, Stenotroph. maltophilia CL-1, B. cereus TB-1, Ent. cloacae KB-2, and Ent. ludwiqii TB-2 had a positive effect on the growth activity of wheat plants for the studied isolates. The data is shown in Figure 11.

Thus, as a result of screening of collection strains and isolates selected from the rhizosphere of wheat soil, it was found that the metabolites of the following crops exhibit the highest activity: B. pumilisPolP3(1) 10 RKM 0528, B.licheniformis 356 RKM 0074, B. subtilis Zb 52 RKM 0285, B.Thuringienspb 30 RKM 0341, Rhiz. legiminosarumB-6 RKM 0272, Rhyz. Leguminosarum RKM 0501, Rhiz. pusenseZ-5 RKM 0517, Azot.ChrococcumAzp 24 V-RKM 820, St. ambofaciens 40 RKM 0736, Agrobact. tumefaciensZ-9 RKM 0520, Agrobact. rhizogenesM-4 RKM 0613, Azot. Chrococcum C9 B-RKM 783, Ps. alcaligenes H-15 RKM 0160, Ps.aeruginosa 36K RKM 0255, Ps. aeruginosa G13 RKM 0417, Ps. putida Vg-84RKM 0060, Ps. aeruginosa Zb 32 RKM 0284, Ps.aeruginosa G23 RKM 0286, Serratia marcescens Sh-2, Serratia marcescens Sh-1, Pseudomonas fluorescensAK-4, Enterobacter ludwigii TB-1, Enterobacter Cloacae KB-2, Enterobacter ludwigii TB-2, Enterobacter cloacae ShB-2, Enterobacter cloacae T-3. In the future, these cultures of microorganisms were selected to form consortia and investigate the additive effect on seed germination and growth activity of wheat plants.

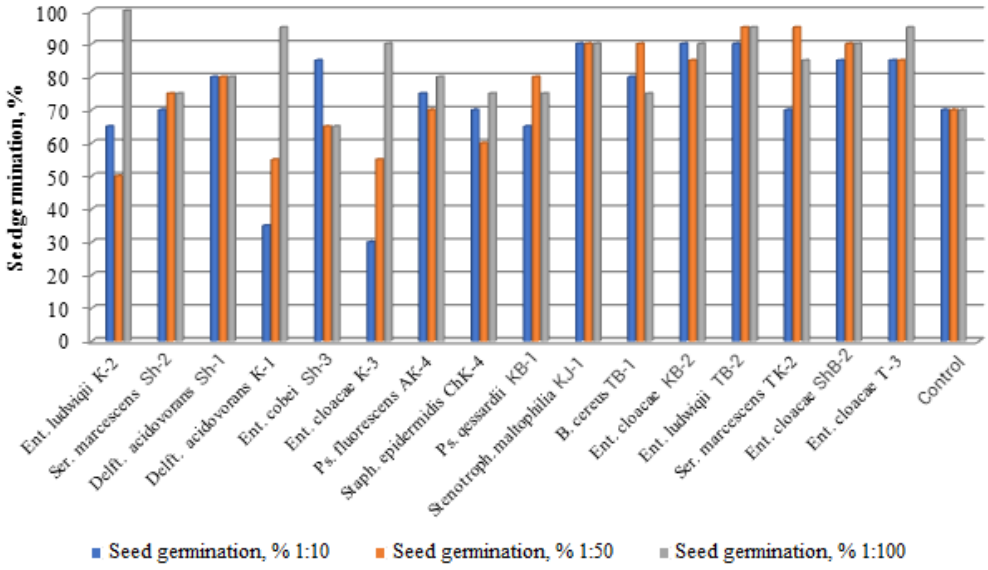

Figure 10. Effect of culture liquid of isolates on wheat seed germination

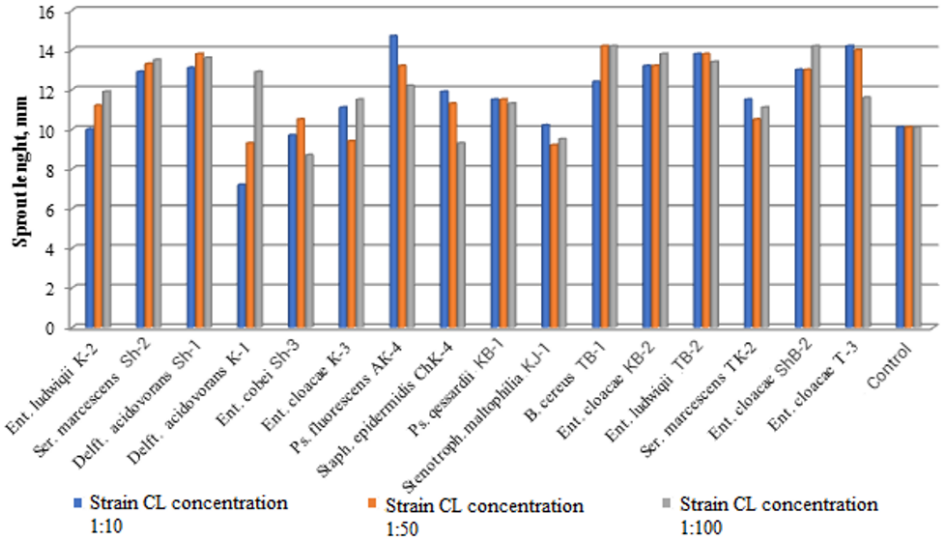

Figure 11. Effect of the culture liquid of isolates on the length of the sprout of wheat plants

3.3 Creation of consortia of microorganisms

Based on the results obtained, microorganisms that were part of the consortia were selected from the collection microorganisms and isolates. Thus, 3 consortia of microorganisms were formed:

1. Consortium No. 1 - Bacillus pumilis Pol P3(1) 10 RKM0528, Bacillus thuringiensis Pb 30 RKM0341, Bacillus licheniformis 356 RKM 0074, isolate Serratia marcescens Sh-2.

2. Consortium No. 2 - Rhizobium legiminosarum B-6 RKM 0272, Azotobacter chrococcum Azp24 B-RKM 820, Bacillus pumilis Pol P3(1) 10 RKM 0528, isolate Serratia marcescens Sh-1.

3. Consortium No. 3 -Rhyzobium leguminosarum RKM 0501, Azot. chrococcum C9 B-RKM 783, Pseudomonas alcaligenes H-15 RKM 0160, isolate Enterobacter cloacae KB-2.

The consortia were formed taking into account the cultural and biochemical properties of microorganisms. In addition, the possibility of joint cultivation was investigated for consortia. The effect of metabolites on wheat seed germination and growth indicators was studied for each of the consortia. The highest results in terms of growth indicators and the percentage of germination of seeds were shown by consortia No. 1 and 2. Thus, for consortium No. 1, with dilutions of culture liquid 1:10, 1:50, and 1:100, the results of the percentage of germination of seeds were 80%, 90%, and 75%, which exceeds the control indicators of 70%. For consortium No. 2, the percentage of seed germination was 80%, 85%, and 70% for dilutions of 1:10, 1:50, and 1:100, respectively. The results are shown in Figure 12. For consortium No. 3, with all three dilutions, the percentage of germination of wheat seeds was less than the control one, which is the result of inhibition of germination by metabolites of these microorganisms. In addition, a complex effect of the interaction of active substances in the culture liquid and their further biological activity is possible, which inhibits the process of germination of wheat seeds.

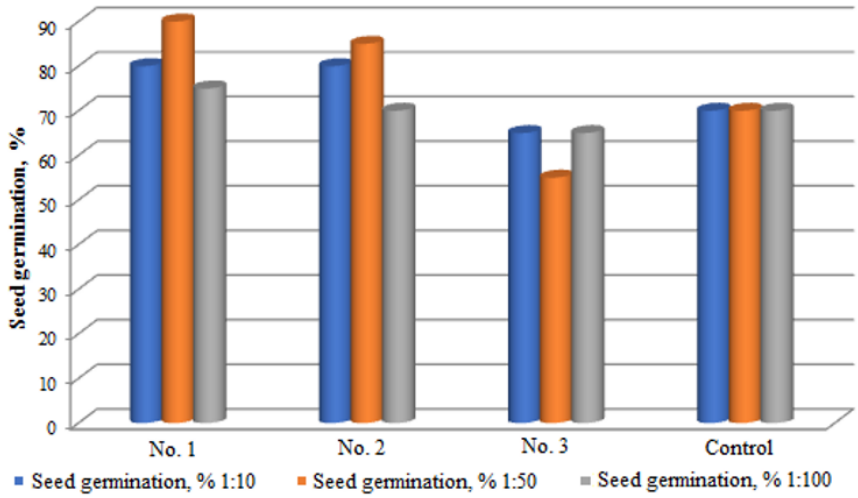

Figure 12. Influence of consortium culture liquid on wheat seed germination

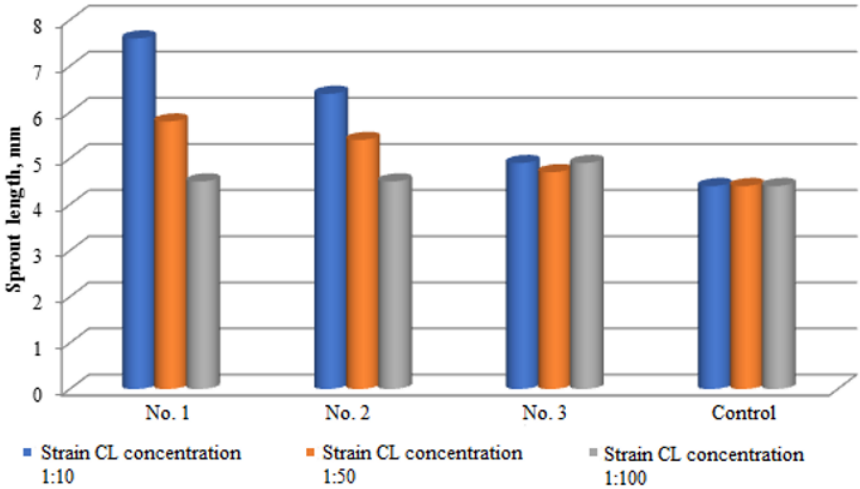

Figure 13. Influence of culture liquid of microbial consortia on the length of wheat plant germ

When studying the effect of the culture liquid of three consortia, the consortia No. 1 and 2 had the greatest positive effect on growth indicators, which coincides with the results of the seed germination study. Thus, for consortium No. 1, the sprout length indicators for dilutions of 1:10, 1:50, and 1:100 corresponded to 7.6, 5.8, and 4.5 mm, with control indicators of 4.4 mm. For consortium No. 2 for the corresponding dilutions of the culture liquid, the length of the sprout corresponded to 6.4, 5.4, and 4.5 mm, with control indicators of 4.4.mm. The data is shown in Figure 13.

Thus, of the three consortia in which the collection strains of microorganisms and isolates were selected, the most significant effect on the growth rates and the percentage of germination of wheat seeds had the culture liquids of consortia No. 1 and No. 2. The rhizosphere, as a zone of close proximity of the soil to the roots of plants, has the property of feedback so that the qualitative composition of microbiomes is stable. Thus, plant roots secrete metabolites that contribute to the colonisation of this zone by certain microorganisms.

The use of microbial consortia as a growth stimulator for crops, including wheat, has been the subject of several comparative studies in recent years. A study [19] compared the effects of three different microbial consortia on wheat growth and nutrient uptake. The researchers found that all three consortia significantly increased wheat growth and nutrient uptake compared to the control group, but that one of the consortia was particularly effective at increasing plant biomass and nutrient content. Another study [20] compared the effects of a commercial microbial inoculant, a bacterial consortium, and a fungal consortium on wheat growth and yield. The researchers found that all three treatments increased wheat yield compared to the control group, but that the commercial inoculant was the most effective at increasing yield and improving wheat quality. A study [21] compared the effects of two different microbial consortia on wheat growth, yield, and nutrient uptake. The researchers found that both consortia significantly increased wheat growth and yield compared to the control group, but that one of the consortia was more effective at increasing nutrient uptake and improving soil quality.

In recent years, such a concept as "rhizosphere engineering" has appeared, which includes screening and investigation of the mutual effect of certain microorganisms, including their consortia and a variety of plants [22, 23]. In most cases, such studies are carried out in laboratory conditions, on a limited amount of material, so the prospect of the study is to investigate the effect of microorganisms on the growth and development of plants in the field conditions [24].

Another important point in the study of the influence of microorganisms on the growth and development of plants and the formation of consortia is the aspect of biosafety [25, 26]. Thus, the microorganisms in the consortia must comply with

biosafety standards, must not only be pathogenic to humans, but also safe for humans and the environment. Microorganisms that are used to improve plant productivity or protect them from pathogens should not be the cause of changes in the soil microflora, since such changes have an impact on the entire ecosystem. That is why the selection of microorganisms based on the already existing representatives of the microbiome of the rhizosphere is a condition that meets the criteria of biosafety.

4. CONCLUSIONS

The greatest contribution of this study is that the influence of the culture liquid of 36 collection strains and 16 isolates of microorganisms from the rhizosphere of wheat soil on the growth indicators of wheat plants and the process of seed germination was investigated. From the analysed individual cultures and isolates, microorganisms were selected that showed the greatest effect on growth indicators and seed germination. Based on the results obtained, 3 consortia of microorganisms were formed. The highest efficiency in relation to the germination of wheat seeds and the effect on growth indicators was shown by the culture liquids of microorganisms that are part of consortia No. 1 and No. 2.

The limitations of the study are that the specific mechanisms of the action of metabolites on plant growth and research in field conditions were not studied. The prospects for further study are to investigate the influence of consortia on other plant cultures and on the microbiomes of the rhizosphere. In addition, an important stage of further work is the study of the mechanism of action of specific substances that make up the culture liquid, these consortia on the growth and development of plants. In the future, such substances can be isolated and studied separately.

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