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Peculiarities of *Bacillus Subtilis* strains influence on the development of *Triticum Aestivum* L. in inoculative cultures

Abstract. The research relevance is predetermined by the need to study strains of *Bacillus subtilis* bacteria that have a positive effect on plant growth and development and exhibit a stimulating effect at optimal bacterial loads. The research is of great importance for agriculture, ecology, and sustainable development. The research aims to determine the effect of new strains of *B. subtilis* (H3, H10, H13, H36, H38, H40, H43, H45) on the development of winter wheat (*Triticum aestivum* L.) seedlings when inoculated with inoculated crops. Laboratory, vegetation, and mathematical and statistical methods were used in the study: deep cultivation of strains, roll method of germination of test plant seeds, and data processing using Statistica 8.0 and MS Excel. The results of the model experiment on the effect of culture liquids of *B. subtilis* strains under different technological forms and dilutions on the growth and development of wheat test plants are summarised. It has been established that at dilutions of 1:10, 1:50, 1:100, and 1:500, the stimulating effect of bioagents is observed, and the maximum effect is achieved at a dilution of 1:100. It has been shown that the greatest positive effect on wheat seed germination was observed for *B. subtilis* inoculants applied to seeds in mature technological forms (spore culture, 2.0×10^7 cells per seed). The germination energy of *Triticum aestivum* L. seeds increased by 96.5% when interacting with *B. subtilis* inoculants, and the raw weight of seedlings increased by 84.0-109.6% depending on the experiment variant compared to the control, which indicates the

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growth-stimulating properties of the new strains. It was proved that the use of mature cultures of *B. subtilis* H38, H40 and H45 resulted in an increase in root weight by 4.8-11.3% compared to the control without bacterisation. When treated with culture fluids of *B. subtilis* H3, H10, H13, H36, and H43 in the form of vegetative cells, the root mass decreased by 11.8-44.0% compared to the control. The use of the studied strains of *B. subtilis* effectively affects the development of winter wheat and is a promising inoculant with a growth-stimulating effect. The practical significance of the study is to understand and determine the potential benefits of using *Bacillus subtilis* bacterial strains to enhance the growth and development of plants, in particular winter wheat (*Triticum aestivum* L.)

Keywords: bacterial inoculant; plants; seeds; winter wheat; growth; stimulating properties

INTRODUCTION

Sustainable development of crop production and increased crop yields in the face of climate change are possible through the use of highly effective biological products based on active strains of microorganisms. The action of the products promotes the synthesis of natural antidepressants and anti-stress factors of resistance to drought, waterlogging and other adverse environmental conditions in the soil and directly in the plant itself (Verma *et al.*, 2018; Vorobei & Logosha, 2021). Biological products inhibit pathogenic microbiota and form an "induced resistance system" in plants, which prevents the development of diseases from phytopathogenic organisms (Gadzalo *et al.*, 2019).

The creation of inoculants consisting of associations of bacteria with additive and synergistic effects on plants and increasing the resistance of the plant-bacterial system is very promising. In addition, when using different inoculants, it is necessary to calculate the bacterial load on the seed, as well as the technological parameters of the formulations selected for use (Borko *et al.*, 2022).

S.F. Kozar (2021) showed a positive synergistic effect of the introduction of mixed cultures of soil microorganisms into various agrocenoses, which is promising both for physiological indicators of plant growth, activation of the biological system "soil-plant-microbiome", and for the possibility of soil improvement, reducing the pesticide burden on the environment (Borko *et al.*, 2022). Due to targeted selection and optimisation of production technologies for the manufacture of modern microbial preparations, beneficial soil microorganisms – the basis of biological products – acquire higher activity, reproduction rate, resistance to temperature fluctuations, light intensity, etc. A promising scientific and practical area of agricultural microbiology is the formation of multifunctional associations of microorganisms that, when introduced into the metagenome of native soil communities, would return non-commodity crop products to biological cycles, and improve soil properties, increasing their fertility (Kopylov *et al.* 2020). The analysis of competitiveness to anthropogenic stress factors creates opportunities for the creation of innovative knowledge-intensive developments for the management of biological processes in agrocenoses. Thus, the selective formation of the diversity of the soil and rhizosphere microbial complex is important for increasing competitiveness and research of complex complexes of plant physiological characteristics.

To ensure the effective introduction of soil microorganisms into crop agrocenoses in the form of various preparative forms, it is necessary to consider several aspects related to the characteristics of the producer strain, the peculiarities of its interaction, the preservation of stability and viability for a long period, as well as the peculiarities and technology of growing a particular crop into the root zone of which beneficial microorganisms are introduced. The methods of seed inoculation used for research purposes are often impossible to use on an industrial scale, and there are significant technical problems with maintaining viable microbial inocula on seeds during seed processing and storage, as noted in several studies by I.K. Kurdish (2018). Other studies show the positive effects of seed inoculation with *Bacillus* bacteria, such as

A.F. Santos *et al.* (2021), where scientists concluded that an inoculant based on a composition of *Bacillus subtilis* and *B. megaterium* is effective for grain yields, as well as for increasing protein content and improving plant fibre digestibility. In other studies, V.F. Guimarães *et al.* (2021) showed that by using similar inoculants, high efficiency can be achieved in the cultivation of legumes, resulting in productivity statistically higher than the control without inoculation.

Most bacteria (including spore-forming bacteria of the genus Bacillus sp.) released from rhizosphere soil form stable associations, which allows microorganisms to gain several competitive advantages when introduced into the root zone of plants (Mawarda *et al.*, 2020). As a result of the introduction, biological agents are exposed to abiotic and biotic factors that can have both positive and negative effects on bacterial activity. Given the current research on understanding the relationship between rhizobacteria and plants, J. Poveda & F. González-Andrés (2021) and T. Tsotetsi *et al.* (2022) summarise the current knowledge base on the key metabolites released by both bacteria and plants, their role in the interaction between organisms and responses to various environmental stresses.

According to scientists, bacteria from the *Bacillus* genus produce secondary metabolites and can be used as natural insecticides for the biocontrol of pathogens through the production of compounds with aseptic activity, synthesis of enzymes that lysate fungal cell walls, and induction of systemic reactions in host plants, and have a positive impact on the soil ecosystem (Saxena *et al*, 2020), enhance plant growth and development through the production of phytohormones, increase the availability of essential nutrients (nitrogen, phosphorus, iron), and increase ethylene levels through ACC deaminase (Oleńska *et al.*, 2020).

Studies by H. Li *et al.* (2021) show that biostimulation of maize crops with *Bacillus sp.* MGW9 strain improves seed germination on saline soils and increases the length of the main root by 7% due to better water uptake, higher chlorophyll, proline, soluble sugar, superoxide dismutase, catalase, peroxidase and ascorbate peroxidase activity, while reducing malondialdehyde content.

In this regard, the search for highly active and new strains of soil microorganisms is relevant for microbiology, biotechnology, soil indication and diagnostics, as well as for screening producers of physiologically active substances.

The research aims to determine the peculiarities of the influence of new strains of *B. subtilis* on the development of test plants (winter wheat seedlings *Triticum aestivum* L.) in the case of using inoculated bacterial cultures.

LITERATURE REVIEW

The gram-positive bacterium *Bacillus subtilis* has a powerful extracellular protein secretion system and does not release any toxic metabolic by-products into the environment. A variety of metabolic processes, and genetic and biochemical variability have led to the widespread use of *B. subtilis* bacteria in various fields of agriculture, medicine, industry (GRAS status – *generally regarded as safe*), and in the production of probiotics, immunoreactive factors, enzymes, amino acids, vitamins, etc. It has been proven to increase the decomposition of plant residues (Avdeeva *et al.*, 2016) and the resistance of barley crops to disease development (Kriuchkova & Patyka, 2020).

Significant efforts of scientists are aimed at both studying and developing ways to control and change the metabolism, gene expression and protein activity of this bacterium (Cui *et al.*, 2018; Chuiko *et al.*, 2021).

Spore-forming bacteria *B. subtilis* (as well as other representatives of the *Bacillus* genus – *B. megaterium, B. atrophaeus, B. licheniformis, B. amiloliquefaciens, B. pumilus, B. mojavensis*, etc.) are among the most sensitive and dynamic components of soil microbial communities, especially under anthropogenic load. Today, research is relevant to study the most active producers of metabolites that have a positive effect on plants – bacteria *B. subtilis, B. pumilus, B. brevis, B. megaterium*, etc., which promote plant growth through the production of phytohormones, dissolution of inorganic phosphates, synthesis of organic acids, antagonism to phytopathogenic micromycetes, etc. For example, under the influence of phytohormone-producing bacteria, changes in the endogenous hormonal balance of plants can occur (Patyka *et al.*, 2019).

A. Shrestha *et al.* (2014) showed that the adhesion and formation of biofilms by soil bacteria on plant surfaces has a phytopathogenic effect and helps to improve root-soil contact. Thus, the ability of bacteria to form biofilms on plants may be a promising area in agriculture to increase plant productivity.

Bacterisation with soil microorganisms has a positive effect on plant biometrics (height, plant length, root system formation) and dry biomass (Kots *et al.*, 2016). The decrease in inoculation efficiency due to the early application of soil microorganisms can be compensated by using a complex of organic substances (e.g., polysaccharide-protein complex as a stability matrix), which increases the viability of microorganism cells in a resting state. Scientists continue to deepen their knowledge of the regularities of the spatial, taxonomic and functional structure of plant-microbial communities in the root zone of plants and the processes underlying plant-microbial interactions (Krutylo *et al.*, 2017), as well as collect modern monitoring data on the soil microbial biome and carry out comprehensive work on analytical selection of soil microbial strains.

Using metabolomic profiling, they detected and identified molecules produced in roots and root hairs during their infection with rhizobia. Their analysis of soybean root hairs suggested that root hairs synthesise flavonoids, amino acids, fatty acids, carboxylic acids, and various carbohydrates in response to inoculation with the above bacteria, with a high content of trehalose (known as an osmoprotective sugar). To date, several compounds have been identified that are involved in various plant-microbe interactions. For example, g-amino butyric acid, hexahydrohexaoxybenzene-O-methyl, glutamine, and lauric acid, help bacteria overcome the plant's defences during infection. These isoflavonoids and fatty acids appear in free form mainly due to their signalling role in symbiosis, which requires the enhancement of cellular biosynthesis to create structural components necessary to support the development of symbiosis.

For the rational use of the potential of regulatory (signalling) plant-microbe interactions, it is necessary to pay attention not only to the effectiveness of new strains and their compatibility but also to know under what conditions inoculant strains can successfully compete with representatives of soil populations of specific bacteria and colonise the root system of plants.

MATERIALS AND METHODS

The research was conducted at the National University of Life and Environmental Sciences of Ukraine, Department of Soil Science and Soil Protection named after Professor M.K. Shykula. In a model laboratory experiment, the effect of bacterization of *Triticum aestivum* L. plants with strains H3, H10, H13, H36, H38, H40, H43, and H45, which in previous studies were isolated from the phylloplane and rhizosphere of the root system of winter wheat and typical black soil, identified and assigned to the genus *Bacillus* sp. *B. subtilis* (Honchar *et al.*, 2021).

Determination of the optimal load of *B. subtilis* bacteria on wheat seeds was carried out in a laboratory experiment. Bacterial inoculants in the form of a suspension of *B. subtilis* cultures were used both in the form of vegetative cells – the beginning of spore formation and complete sporulation (mature culture). *B. subtilis* strains were cultured on Luria-Bertani (LB) liquid nutrient medium for 72 hours in an orbital shaker incubator at a temperature of at least 28-30°C according to generally accepted methods in microbiology (Tepper *et al.*, 1979; Goldman & Green, 2021). There are treatment options with the following bacterial load: 1) 2.0×10^8 cells/1 seed; 2) 2.0×10^7 cells/1 seed; 3) 2.0×10^6 cells/1 seed; 4) control. Replication was performed four times.

The effectiveness of bacterial inoculants *B. subtilis* on the growth and development of *Triticum aestivum* L. was studied in a model experiment. The scheme of the experiment included: 1 – control (without bacterisation); 2-9 (treatment with the appropriate strain of spore-forming bacteria *B. subtilis* in the form of vegetative cells); 10-17 (treatment with the appropriate strain of *B. subtilis* bacteria in the form of technological maturity of the crop with complete spore release). In the experiment, the seeds of *Triticum aestivum* L. were germinated by the roll method. Two layers of moistened filter paper in the form of ribbons measuring 10×55 cm were used, on which seeds were laid out with the embryos down in one line at an interval of 1 cm and at a distance of 2.0 cm from the side edges and surface of the ribbons (70 pieces in one row). The seeds laid out on the paper were covered with moistened filter paper and polyethene tapes of the same size and rolled loosely. The wrapped rolls were placed vertically in beakers with *B. subtilis* culture fluid and placed in a thermostat at 23-250°C (DSTU 4138-2002..., 2004). The water level in the beakers was monitored to prevent drying out, and distilled water was added to the beakers with the rolls if necessary (water change every 3-5 days). The germination rate of the test culture (length of roots and seedlings) on day 10 was determined using a conventional centimetre scale. Statistical analysis was performed using Statistica 8.0 software, data were calculated using MS Excel.

Experimental studies of plants (both cultivated and wild), including the collection of plant material, were carried out following the requirements of the Convention on Biological Diversity (1992).

RESULTS AND DISCUSSION

The main principle in the treatment of agricultural plants with microbial preparations with growth-stimulating activity is the need for a detailed study and establishment of the physiologically appropriate time for plant treatment (developmental stage) and determination of the rational inoculation load when applying them.

As a result of biotesting and studying the effect of the culture fluid of new *B. subtilis* strains on the growth and development of wheat plants, it was found that at dilutions of 1:10, 1:50, 1:100, and 1:500, the stimulating effect of bioagents was observed (Fig. 1). Thus, the lowest stimulatory effect of *B. subtilis* strains H3, H10, H13, and H36 was observed at a dilution of the culture fluid of 1:500, and the maximum stimulatory effect was observed at a dilution of 1:100. This confirms that the biological efficacy of *B. subtilis* bacteria is largely due to the production of auxin, cytokinin and gibberellin hormones, and hormonal substances synthesised by microorganisms are extracellular and are present directly in the culture fluid of the strains.

Thus, the study is confirmed by C. Accinelli *et al.* (2018), where the treatment with beneficial microorganisms *B. subtilis* increased shoot length (+7%) and root length (+10%) in maize for grain. The method used to achieve the positive effect was the incorporation of the bacteria into a bioplastic-based composition, rather than the use of biofilms or dilutions.

G.M. Teixeira *et al.* (2021) revealed a positive effect of the use of bacterial biostimulation of soybean seeds, which increased germination rate by 15%, root length by 33% and root mass by 27% compared to the control.

The results are generally positive but variable, so more scientific information is needed for different crops and cultivation technologies, considering different beneficial microbes (species and strains) and variable climatic conditions, to understand the impact of seed treatment.

Reports on studies of metabolites of soil microorganisms or physiologically active substances of microbial origin, which are more appropriate for regulating plant growth and development, can be found in studies (Kots' *et al.*, 2016).

The native culture fluid of *B. subtilis* strains H10, H13, H40, H43 and H45 was found to inhibit the growth of coleoptiles, the rate was lower than the absolute control by 17.0-35.0%. The results obtained indicate the high activity of strains producing auxin-like compounds in the culture fluid and the feasibility of using a diagnostic test for the auxin activity of *B. subtilis* exometabolites in studies.

In general, the dilution of the culture fluid had a positive effect on the growth of wheat coleoptiles (Fig. 1), which indicates the production of physiologically active compounds in different concentrations by *B. subtilis* strains (a manifestation of the stimulatory effect). The exogenous supply of active metabolites to plants under the introduction of *B. subtilis* can also contribute to the optimisation of organogenesis processes and, as a result, increase crop productivity.

It is worth noting that liquid forms of microbial preparations make it possible to apply them with the help of special sprayers during the vegetation of plants in certain phases without additional manipulations. The optimal combination of pasteurisation and treatment of vegetative plants, considering the appropriate rates of preparation and the timing of treatments, is considered extremely important for the development of an effective method of applying microbial preparation. Following scientific, theoretical and practical studies, it has been proven that the signalling interaction between plants and microorganisms is associated with the regulatory functions of plants (especially concerning the rate of reproduction and the number of bacterial cells). Such regulation is very important because the potential rate of reproduction and metabolic activity of bacterial cells is much higher than that of plant cells. Therefore, the interaction of plants and microorganisms is a complex process that is implemented through molecular mechanisms and metabolic integrations (Patyka *et al.*, 2019).

It is known that the morphometric parameters of the growth and development of cultivated plants do not change significantly depending on the method of using microbial preparations, both when treated with the culture fluid of the producer strain and the supernatant of the bacterial culture fluid (O'Callaghan, 2016; Gadzalo *et al.*, 2019). Scientists from around the world have shown that such treatments equally contribute to the improvement of biometric parameters of the culture. In addition, the formation of growth parameters of agricultural plants depends on the rate of application of the microbial preparation. Microbiological regulation of growth processes is an effective means of increasing the resistance of cultivated plants to adverse environmental factors and increasing crop productivity (Pandey *et al.*, 2018) by mobilising the potential capabilities of the plant organism.

The study of O. Lastochkina *et al.* (2020) confirms the results (Fig. 1) and proves the protective effect of Bacillus subtilis (strain 10-4) against drought stress. The bacterium was applied by biopriming to wheat seeds that were either sensitive (T. aestivum cv. Salavat Yulaev) or tolerant (cv. Ekada 70) to drought conditions during the germination phase. B. subtilis promoted germination and plant growth of 6-day-old seedlings (both length and fresh/dry weight of roots and shoots) under normal growth conditions and rapidly activated specific metabolic adaptations to drought stress conditions by reducing lipid peroxidation, proline content and electrolyte leakage in 21-day-old seedlings.

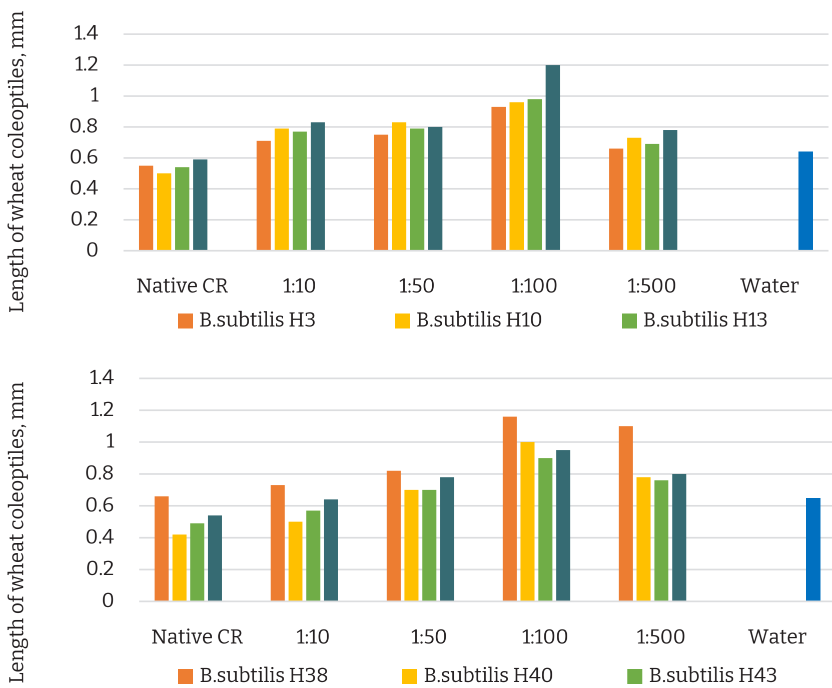

Figure 1. The activity of physiologically active metabolites of *B. subtilis* strains and their influence on wheat coleoptiles growth

As a result of the studies, the complex parameters of stimulation of *B. subtilis* bacteria were determined by analysing the germination parameters of the test crop *Triticum aestivum* L., namely: energy, germination, speed, and friendliness of germination, as well as seedling weight and root weight (based on the cell load per seed). The most positive effect on wheat seed germination was observed for *B. subtilis* inoculants applied to seeds in mature technological forms (spore culture) and at the rate of 2.0×10^7 cells per seed (Table 1).

Table 1. Biometric indices of germination of the test culture *Triticum aestivum* L. under the influence of inoculation cultures of spore-forming bacteria *B. subtilis*, model experiment

Experiment variants	Germination energy, %	Root system length, cm	% to control	Length of seedlings, cm	% to control
for the use of vegetative cells					
Control (water treatment)	89.0±1.4	12.7±1.9	-	9.8±2.6	-
B. subtilis H3	90.2±2.8	13.6±1.9	107.0	9.0±2.0	91.8
B. subtilis H10	90.6±2.4	12.0±1.9	94.5	8.6±2.1	87.8
B. subtilis H13	90.0±2.8	12.2±1.7	96.1	8.3±2.0	84.7
B. subtilis H36	90.0±2.8	14.3±1.6	112.6	10.4±1.6	106.1
B. subtilis H38	89.3±1.4	14.0±1.6	110.2	8.5±2.0	86.7
B. subtilis H40	89.7±1.4	12.8±1.9	100.8	9.6±2.0	98.0
B. subtilis H43	90.5±2.8	13.0±1.7	102.4	11.5±3.5	117.3
B. subtilis H45	91.0±2.4	14.0±1.6	110.2	11.2±3.5	114.3
for using a mature spore culture					
B. subtilis H3	90.7±2.8	14.3±1.6	112.6	11.8±1.9	120.4
B. subtilis H10	96.0±1.5	14.8±1.7	116.5	11.5±1.6	117.3
B. subtilis H13	96.4±1.5	13.7±1.9	107.9	11.6±1.6	118.4
B. subtilis H36	96.5±1.5	14.1±1.1	111.0	12.0±1.8	122.4
B. subtilis H38	96.2±1.5	13.8±1.9	108.7	12.3±2.0	125.5
B. subtilis H40	90.7±2.8	13.5±2.3	106.3	10.3±1.6	105.1
B. subtilis H43	93.5±3.8	14.3±1.6	112.6	12.0±1.8	122.4
B. subtilis H45	91.7±2.4	14.5±1.6	114.2	12.2±3.5	124.5

Source: compiled by the authors

The positive effect of microbial preparations based on *Bacillus subtilis* sp. on the biometric parameters of winter wheat plants was also noted in the works of other authors (Liu *et al.*, 2017). Several scientists have described the positive effect of both synthetic growth stimulants (Calvo *et al.*, 2014) and microbial preparations with growth-stimulating effects (Yadav & Chandra, 2014) on plant growth and development. However, microbial preparations are usually used classically, namely for seed bacterisation.

The laboratory germination rate of winter wheat seeds was more than 92.5% and tended to actively form future seedlings and roots. In general, the germination rate of the test crop met the requirements for the seed. A slight decrease in seed germination under the roll method is compensated by its rapid germination, higher seedling formation index and relatively better growth and development rate.

The germination energy of winter wheat (*Triticum aestivum* L.) seeds increased by 96.5% when using *B. subtilis* inoculant bacteria. It should be noted that the use of spore-forming bacteria activates the growth of primary roots of winter wheat plants and, in general, increases the length of the root system (by 6.3-16.5%, respectively, compared to the variant without treatment with the strains).

The treatment of the biotest with inoculated cultures of *B. subtilis* led to an increase in the raw weight of seedlings by 84.0-109.6%, depending on the experiment variant, compared to the control (Table 2), which indicates the growth-stimulating properties of the new strains.

The data obtained indicate the biological characteristics of the experimental strains of *B. subtilis*, which are associated with the formation of effective plant-microbe interactions through the exchange of exometabolites. The mechanisms of interaction between bacteria and plants are controlled by both partners and provide them with mutual benefit. This interaction results in the stimulation of plant growth and development and stabilisation of its production process.

It is known that the inoculation of plants with cytokinin-synthesising bacteria stimulates the accumulation of biomass of both shoots and roots (Liu *et al.*, 2017). According to the studies, it was found that the mass of roots using *B. subtilis* cultures in the form of vegetative cells (*B. subtilis* strains H3, H10, H13, H36, H43) was 11.8-44.0% lower than in the control. The use of mature spore culture of *B. subtilis* (*B. subtilis* H38, H40 and H45) resulted in an increase in root weight by 4.8-11.3% compared to the control variant without bacterisation. Thus, the effect of the studied inoculant strains on the weight of the primary roots of test plants was unequal, and bacterisation of *Triticum aestivum* L., in general, promotes better development of the root zone of test plants (which may further affect the activation of the adsorption capacity of roots to absorb nutrients from the substrate medium, soil) (Table 2).

Table 2. Influence of *B. subtilis* inoculation cultures on the wet weight of seedlings and roots of *Triticum aestivum* L.

Experiment variants	Raw weight of roots, mg	% to control	Raw weight of seedlings, mg	% to control
for the use of vegetative cells				
Control (water treatment)	85.0±3.6	-	103.5±6.3	-
B. subtilis H3	75.0±10.4	88.2	110.0±4.1	106.3
B. subtilis H10	55.5±4.5	65.3	89.0±18.0	86.0
B. subtilis H13	56.0±4.5	65.9	87.3±18.4	84.0
B. subtilis H36	76.0±7.4	89.4	104.8±6.0	100.5
B. subtilis H38	89.0±3.4	104.7	102.7±5.8	99.2
B. subtilis H40	87.5±14.4	102.9	102.5±5.5	99.0
B. subtilis H43	76.4±7.0	89.9	108.5±5.1	104.8
B. subtilis H45	94.0±9.8	110.6	107.8±8.6	104.2
for using a mature spore culture				
B. subtilis H3	76.3±7.0	89.8	110.2±4.0	106.5
B. subtilis H10	75.8±11.1	89.2	105.3±6.6	101.7
B. subtilis H13	79.0±5.7	92.9	105.5±6.6	101.9
B. subtilis H36	83.2±3.6	97.9	113.0±4.1	109.2
B. subtilis H38	90.0±9.6	105.9	113.4±4.1	109.6
B. subtilis H40	89.1±3.4	104.8	106.0±5.1	102.4
B. subtilis H43	80.3±3.7	94.5	110.5±4.0	106.8
B. subtilis H45	94.6±5.9	111.3	110.0±4.1	106.3

Source: compiled by the authors

Summarising the results of the research, it can be noted that new strains of *B. subtilis* in the form of inoculated cultures had a positive effect on the development of winter wheat *Triticum aestivum* L., which indicates the production of extracellular hormonal substances by bioagents in different concentrations, the so-called stimulatory effect. The mature technological forms of *B. subtilis* inoculants accelerated the germination of wheat seeds and activated the growth of primary plant roots and the formation of their root system. The growth-stimulating properties of the new *B. subtilis* strains were also confirmed by the increase in the biomass of both seedlings and plant roots.

CONCLUSIONS

The signalling response of plants to bacterial inoculants *in vivo* depends on several environmental factors, including plant genotype, which can significantly reduce the efficiency of their practical use. However, pre-sowing seed treatment remains the most affordable and effective agricultural measure today. Since plant-microbial interaction (colonisation of the rhizosphere, plant phylloplane; production of antimicrobial metabolites, physiologically active, phytohormonal substances of auxin, gibberellin, cytokinin nature and vitamins; induction of systemic resistance in the plant, etc.) is considered an important mechanism of biological control of agricultural systems.

Following research results, it is shown that the data obtained at the level of model testing of *B. subtilis* bioagents on winter wheat (*Triticum aestivum* L.) plants allow us to identify the positive effect of inoculants associated with the main indicators of plant growth and development and to trace the stimulating effect when using optimal dilutions of the culture fluid. The exogenous supply of active metabolites to plants upon introduction of *B. subtilis* strains makes it possible to optimise the processes of organogenesis. It was found that at dilutions of 1:10, 1:50, 1:100, 1:500, the stimulating effect of bioagents was observed: from minimal (*B. subtilis* strains H3, H10, H13, H36 in the case of dilution of the culture fluid 1:500, to the maximum stimulating effect at dilution 1:100). Thus, the biological efficacy of *B. subtilis* strains is largely due to the production of exometabolites that are concentrated in the culture medium. At the same time, the native culture fluid of *B. subtilis* strains H10, H13, H40, H43 and H45 inhibits growth processes, which indicates the activation of auxin-like compounds producers in the culture fluid. In the analysis of the effectiveness of plant-microbial interaction in the case of inoculation with different technological cultures of *B. subtilis*, the expediency of using mature spore cultures of *B. subtilis* spore cultures (2.0×10^7 cells per seed), which makes it possible to manifest the growth-stimulating properties of new strains, in particular, according to the parameters of the raw weight of seedlings and roots of *Triticum aestivum* L. Thus, the knowledge about the peculiarities of the influence of new strains of *B. subtilis* on the development of winter wheat as promising inoculants with the effect of growth stimulation has been expanded.

The search for new producer strains with a protective and stimulating effect and the study of their peculiarities of influence on the plant organism is a promising scientific direction. This will reveal new patterns of spore-forming bacteria and their role in the nutrition of cultivated plants and is also necessary for improving microbial inoculants to increase their manufacturability and efficiency when used in modern crop cultivation technologies.

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CONFLICT OF INTEREST

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Особливості впливу штамів *Bacillus Subtilis* на розвиток *Triticum Aestivum* L. у разі застосування інокуляційних культур

Анотація. Актуальність дослідження полягає у необхідності вивчення штамів бактерій *Bacillus subtilis*, які позитивно впливають на ріст і розвиток рослин та проявляють стимулювальний ефект за оптимальних бактеріальних навантаженнях. Дослідження має велике значення для сільського господарства, екології та сталого розвитку. Мета дослідження полягає у визначенні впливу нових штамів *B. subtilis* (H3, H10, H13, H36, H38, H40, H43, H45) на розвиток проростків пшениці озимої (*Triticum aestivum* L.) у разі застосування інокуляційних культур. В роботі використано лабораторні, вегетаційні та математично-статистичні методи: глибинне культивування штамів, рулонний метод пророщування насіння тест-рослин, обробка даних за програмами Statistica 8.0, MS Excel. Узагальнено результати модельного досліду стосовно впливу культуральних рідин штамів *B. subtilis* за різних технологічних форм і розведень на ріст і розвиток тест-рослин пшениці. Встановлено, що при розведеннях 1:10, 1:50, 1:100, 1:500 спостерігається стимулювальна дія біоагентів, а максимальний ефект досягається при розведенні 1:100. Показано, що найбільший позитивний вплив на проростання насіння пшениці мали інокулянти *B. subtilis*, які наносили на насіння у зрілих технологічних формах (спорова культура, $2{,}0\times10^7$ клітин на насінину). Енергія проростання насіння *Triticum aestivum* L. підвищується при взаємодії з інокулянтами *B. subtilis* до 96,5 %, а також збільшується сира маса проростків на 84,0-109,6 % залежно від варіанту досліду порівняно з контролем, що свідчить про рістстимулювальні властивості нових штамів. Доведено, що за використання зрілих культур *B. subtilis* H38, H40 і H45 відбувається зростання маси коренів на 4,8-11,3 % порівняно з контролем без бактеризації. При обробці культуральними рідинами *B. subtilis* H3, H10, H13, H36, H43 у формі вегетативних клітин маса коренів зменшується на 11,8-44,0 % порівняно з контролем. Використання досліджених штамів *B. subtilis* ефективно впливає на розвиток пшениці озимої і є перспективним інокулянтом з ефектом рістстимуляції. Практичне значення дослідження полягає у розумінні та визначенні потенційних користей від застосування штамів бактерій *Bacillus subtilis* для підвищення росту і розвитку рослин, зокрема озимої пшениці (*Triticum aestivum* L.)

Ключові слова: бактеріальний інокулянт; рослини; насіння; пшениця озима; ріст; стимулювальні властивості