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Microorganisms on granular fertilizers and prospects of their application as PGPM

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ABSTRACT

The study aimed to investigate the degree of microbial contamination in mineral fertilizers, assess the presence of PGPM (Plant Growth-Promoting Microorganisms) among them and evaluate the possibility of using these microorganisms for fertilizer enrichment. The number of microorganisms on fertilizer granules was determined by plating water washes on nutrient media. Bacteria were identified by morphological, physiological, biochemical properties and using the MALDI-TOF method. The effectiveness of mineral fertilizer enriched with bacteria was studied during potato cultivation on sod-podzolic soil. Bacteria were present on fertilizer granules (from 11,000 CFU/g superphosphate to 74,000 CFU/g ammonium sulfate). Micromycetes were not detected. Regardless of the type of fertilizer, representatives of *Bacillus amyloliquefaciens* were dominant on the granules. A small number of *B. amyloliquefaciens* subsp. *plantarum*, *B. cereus* and *Micrococcus* spp. were also detected. A total of 112 bacterial cultures were isolated. Twenty percent of isolates showed a growth-stimulating effect. Phosphate-solubilizing properties were observed in 28% of isolates, and 36% showed antagonistic activity against phytopathogenic micromycetes. Three active bacterial strains were selected for the enrichment of mineral fertilizer. NPK granules (16-16-16) were separately treated with suspensions of *B. amyloliquefaciens* B-22, *B. cereus* 3/7 and *B. amyloliquefaciens* subsp. *plantarum* 5/13. The use of biologically modified fertilizers at a dose of $N_{80}P_{80}K_{80}$ during potato cultivation led to an intensification of the production process, increased crop yield and improved product quality. The results of the study indicate the feasibility of further studying bacteria isolated from mineral fertilizers to determine the prospects for the microbiological enrichment of fertilizers.

Keywords: potato, bio-preparations, mineral fertilizers, microorganisms, yield, sustainable agriculture

INTRODUCTION

Today, the use of agronomically useful microorganisms in crop cultivation technologies is becoming the norm in agriculture in many countries. At the end of the 20th century, the term plant growth-promoting rhizobacteria (PGPR) was introduced to describe isolated rhizospheric pseudomonads that rapidly colonized plant roots and increased yield after seed inoculation (Kloepper and Schroth, 1979; Kloepper et al., 1980). Later, the concept of PGPR was extended to any other bacteria (PGPB) or any microorganism (PGPM) exhibiting plant growth promoting (PGP) properties such as nitrogen fixation, phosphate and potassium solubilization, production of siderophores, phytohormones and 1-aminocyclopropane-1-carboxylate (ACC) deaminase (which facilitates plant growth and development by reducing ethylene levels and reducing stress (salinity and drought) in plants), or which reduce or prevent the harmful effects of phytopathogenic organisms (Beneduzi et al., 2012; Ma, 2019; Volpiano et al., 2022). Based on PGPM-based biological preparations are created, which are increasingly considered as an environmentally sound addition (and sometimes an alternative) to agrochemicals with the potential to solve two global problems – food security and environmental sustainability of agricultural production (Volkogon et al., 2006; Singh and Trivedi, 2017; Lopes et al., 2021). At the same time, the efficiency of PGPM largely depends on the form of the inoculant and the peculiarities of its application (Backer et al., 2018).

The production of inoculants for crops is mainly focused on obtaining suspensions of bacterial cultures (with or without stimulating or stabilizing additives) and on their combination with inert solid carriers, among which peat is the most common (Volkogon et al., 2006; Bashan et al., 2014). Biochar is an alternative to peat, given its porosity (Głodowska et al., 2016). The above forms of inoculants are mainly used for pre-sowing seed inoculation. Liquid inoculants can also be applied locally, directly into the sowing groove, using appropriate mechanisms. In addition to the specified forms of preparations, the use of microencapsulation and immobilization of bacterial cells on polymer matrices (such as alginate, agar, pectin, chitosan, polyacrylamide) has also been proposed (He et al., 2015; Herrmann and Lesueur, 2013). For this purpose, highly dispersed clay materials can also be used (Kurdysh, 2001). After encapsulation, cells are relatively protected by the matrix from mechanical damage and stress. At the same time, the slow degradation 999of the matrix material releases microorganisms for a long time, which theoretically will contribute to the optimization of the formation of close plant-bacterial associations (Bashan, 1986; Strobel et al., 2018; Vejan et al., 2019). Under these conditions, bacteria are also protected from the harmful effects of agrochemicals on seeds and in the soil. Unfortunately, these technologies are quite limited due to the difficulties in their implementation.

Crop growing technologies also use foliar treatment with the use of bioinoculants (Shevchenko, 2017; Berger et al., 2018), which should be considered as a kind of addition to the classical method of seed inoculation. Foliar treatment can be extremely effective using microbial preparations with a high content of phytohormones and other physiologically active substances. Theoretically, the efficiency may outweigh the impact of the use of individual phytohormones or their <šynthetic analogues, since a complex of active factors will act on the plant.

Recently, there have been reports on the efficiency of mineral fertilizers enriched with agronomically valuable microorganisms (Skrypka et al., 2016a, 2016b; Couture and Arshad, 2016; Ahmad et al., 2017; Dzhumaniyazova et al., 2018; Sas-Paszt et al., 2021). Surely, such solutions are quite appealing, given the potential simplicity of the application of PGPM, bypassing the pre-sowing inoculation of seeds of crops. Still, the small number of publications on this array raises many questions, among which, first of all, how reproducible the obtained results are, since the survival of microorganisms on granular fertilizers can be limited by an aggressive chemical environment. Logical enough, the best solution for this is the use of microorganisms capable of sporulation. Despite the aggressive environment, the surface of mineral granular fertilizers can be colonized by random representatives of the microbiota, which in turn can limit the preservation of PGPM applied to the granules. It may also happen that agronomically valuable microorganisms can be found on mineral granular fertilizers. In this case, there is a possibility of their isolation and further use for artificial enrichment of fertilizers. At the same time, their ability to survive in aggressive conditions might be a positive feature.

In connection with the above, the objective of our research was to find out the degree of contamination of mineral granular fertilizers by microorganisms and to determine the conceptual possibilities of the success of using mineral fertilizers enriched with microorganisms in agricultural technologies.

MATERIALS AND METHODS

Microbiological aspects of the research

The number of microorganisms on fertilizer granules was determined by seeding aqueous dilutions of washings from granules. To maximally reduce the negative effect of chemicals on microorganisms that could form toxic solutions when in contact with water, 10 g of fertilizers were placed in flasks with 100 mL of sterile water and shaken for one minute. Extended shaking could contribute to the death of cells of microorganisms and, thus, distort the results. Half of the resulting water washes were pasteurized by heating in a water bath at 70 °C. We used this approach due to the need to account for the number of spore-forming microorganisms.

Granules of the following types of fertilizers were used in the study: Azofoska (N-16; P-16; K-16), polyfoska (N-8; P-24; K-24; S-9), ammonium sulphate (N-21; S -24), urea (N-45), magnesium sulphate (MgO-16; SO_3-32), kaliimag (potassium + magnesium) (K-44; Mg-15; Ca-5; S-3), simple superphosphate (P-19.5). Fertilizers were purchased from various commercial structures that sell them on an industrial scale.

Meat peptone agar (MPA) was used to count bacteria; Czapek medium – to count microscopic fungi (Gerhardt, 1981).

Colonies of microorganisms grown on agarized media were re-sowed into test tubes on slanted agar, and were subjected to microscopy when growth was achieved (microscope Micros MC50). The purity of the isolates was checked by surface sowing on Petri dishes with appropriate media. We described the features of colony formation, nature of growth and development, morphological features of cells, and ability to form spores, thus grouping microorganisms into the appropriate morphotypes.

Bacterial isolates were tested for the ability to produce physiologically active substances, using barley as a test culture (Guidelines for the determination of phytohormones, 1988). For this purpose, the isolates were grown in a liquid citrate medium (g/L): glucose – 10.00; $Na_3C_6H_5O_7 \cdot 2H_2O$ – 1.29; $(NH_4)_2HPO_4$ – 4.75; KH_2PO_4 – 9.60; $MgSO_4 \cdot 7H_2O$ – 0.18; pH = 7.2–7.6 (Grabova et al., 2017), the seeds were soaked for 2 hours in the native culture liquid and its aquatic dilutions (1 : 10, 1 : 50, 1 : 100, 1 : 500, 1 : 1000, 1 : 5000), then placed on moist filter paper in Petri dishes and cultivated at 26 °C. The length of the sprouts was determined.

The ability of microorganisms to dissolve mineral phosphorus compounds was tested by sowing isolates on an agarized digest medium with $Ca_3(PO_4)_2$ (Volkogon et al., 2010). The intensity of cellulose decomposition was measured as a reflection of the activity of hydrolytic enzymes. For this purpose, we used agarized glucose-salt medium with carboxymethyl cellulose (CMC), (g/L): NH_4Cl – 2.0; NH_4NO_3 – 0.4; $Na_2SO_4 \times 10\ H_2O$ – 0.8; $K_2HPO_4 \cdot 3H_2O$ – 1.57; $KH_2PO_4 \cdot 3H_2O$ – 0.56; $MgSO_4 \cdot 7H_2O$ – 0.04; glucose – 0.8; CMC – 2.0; agar-agar – 10.0. At day 5 of cultivation, a 0.1% aqueous solution of Congo red was added to Petri dishes with bacterial colonies. After exposure (10 min), the Congo red solution was washed off the surface of the medium with 8% NaCl solution, and the diameter of the translucent zones (CMC hydrolysis zones) formed was measured (Varbanets and Borzova, 2010).

Furthermore, the antagonistic properties of the isolates against pathogens of certain agricultural crop diseases (*Fusarium culmorum* Sacc. 50716, *F. moniliforme* 156, *F. oxysporum* 311, *F. solani* (Mart.) Sacc. EK 1, *Nigrospora oryzae* Berk. 5000, *N. oryzae* 3000, *Alternaria alternata* 89) were studied. The abovementioned strains of micromycetes are kept in the collection of the Laboratory of Plant-Microbial Interactions of the Institute as test cultures for determining the antagonistic activity of bacteria. Antagonistic properties of bacteria were measured by the dual culture method on potato-sucrose agar (PSA) (Egorov, 2004). To measure the antifungal activity of bacteria by the dual culture method, fungal mycelium was sown in Petri dishes injecting them, and at a distance of 6 cm from the injection, a bacterial isolate was sown by the baco-strip method. Pure fungal cultures sown in separate dishes served as controls. At day 7, the results were recorded. The inhibitory activity was measured using the diameters of the colonies in the control and experimental variants, according to the formula (Dc–Dt)/Dc, where Dc means the diameter of the colony in the control, Dt means the diameter in the experiment, expressing indicators in % (Egorov, 2004).

The nitrogenase activity of the isolates was measured by the acetylene method (Hardy et al., 1968). For this purpose, vessels ("penicillin" vials) with Ashby semi-liquid nitrogen-free medium were inoculated with appropriate microorganisms, after 72 hours the cotton plugs were replaced with rubber ones, acetylene was introduced in the amount of 10% of the volume of the vessels, and after an hour the gas samples were analyzed for ethylene content on the Chrom-5 gas chromatograph.

Pre-identification of isolated bacteria was carried out by morphological and cultural properties (Vos et al, 2009). To clarify the identification, the MALDI-TOF method was used on a MALDI-TOF automatic bacteriological analyzer (Bruker, USA; Welker, Moore, 2011; UK Standards, 2015).

Bacteria with the highest growth-stimulating, antagonistic and phosphate-solubilizing activities, which could potentially have a positive effect on plant growth and development (designated as: *Bacillus* sp. B-22, *Bacillus* sp. 5/13, and *Bacillus* sp. 3/7) were retested and used in subsequent fertilizer enrichment studies. For this purpose, an inoculum was obtained for growing bacteria on MPA for 72 hours at 28 °C, after which the biomass was washed with sterile water. The titre of bacterial suspensions was measured by microscopy using a Horiaiev chamber and by sowing suspension dilutions on MPA. The resulting suspensions were applied to the granules of azofoska. The volumes of suspensions of microorganisms used were 2% of the mass of fertilizer (such a volume was necessary for homogeneous application of microorganisms on mineral granular fertilizers). The fertilizer for the control variants was treated with water. The variants of the enriched azofoska obtained this way were dried for 24 hours at room temperature and applied to the soil according to the design of the field experiment.

Design of the field experiment

The study of the efficiency of mineral fertilizers enriched with microorganisms was carried out in 2023 under the conditions of a field experiment on a sod-podzolic cultivated dusty-sandy soil (pH_{KCl} – 5.8; humus content – 1.1%; easily hydrolyzable nitrogen – 45 mg/kg; P_2O_5 – 170 mg/kg; K_2O – 62 mg/kg of soil) when growing Bellaroza potatoes. Repetition in experiments is sixfold. The area of one plot was 14.7 m^2 (2.1 m × 7 m). Placement of the experimental plots was random. The predecessor of the potato in the experiment was the soybean.

Mineral fertilizers for potatoes were used at the rate of $N_{80}P_{80}K_{80}$. Accordingly, the amount of fertilizers (in physical weight) was 735 g/plot.

Experimental design:

1. Non-enriched fertilizer, control;
2. *Bacillus* sp. B-22-enriched fertilizer;
3. *Bacillus* sp. 5/13-enriched fertilizer;
4. *Bacillus* sp. 3/7-enriched fertilizer.

Fertilizers were applied locally, into the rows when planting potatoes on April 25.

Study of the physiological state of plants, accounting of the harvest and study of the quality of the resulting products

During the potato flowering phase, the content of chlorophylls a and b in the leaves was measured (Grodzinskyi, Grodzinskyi, 1973), the area of the photosynthetic apparatus of plants was calculated (Kaushik et al., 2021), and the mass of the root system of plants in the 0–30 m soil layer was measured by the monoliths washing off method (Böhm, 1979).

The accounting of the potato harvest was carried out by direct weighing from each plot. The total yield of the crop and the mass of marketable tubers were measured. Tubers weighing more than 70 g were considered marketable. The resulting values were converted into yield as t/ha.

The content of starch and nitrates was measured in potato tubers (Ermakov, 1972).

Weather conditions during the experimental year

In 2023, climatic conditions were generally favourable for the vegetation of potato plants and the formation of crop yield, with sufficient moisture (307 mm for the period "April-August") and relatively minor deviations of temperature indicators from the average perennial.

Statistical analysis

Differences between variants in determining the number of microorganisms on granular fertilizers, phosphate-mobilizing, growth stimulatory, and antagonistic activities, as well as product quality indicators, were analyzed using the arithmetic mean value and standard deviation, calculated using the MS Excel Analysis Tool Pak. Potato yield was analyzed using the ANOVA with a probability level of $P<0.05$ using Statistica 6.0 software (StatSoft Inc., USA).

RESULTS

Analysis of the number of microorganisms when sowing dilutions of aquatic washes from mineral granular fertilizers on appropriate digest media shows the absence of growth of micromycetes. The calculation of the number of bacteria on the MPA shows that their number is quite high, 11,000 CFU/g of superphosphate to 74,000 CFU/g of ammonium sulphate (Table 1). Therefore, microorganisms on granules are represented by prokaryotes. Pasteurization reduced the number of bacteria, but did not completely destroy them.

Analysis of bacterial colonies and their microscopy indicates the presence of several morphotypes. Attention is drawn to the dominance of one of the bacterial morphotypes on the granules, regardless of the type of fertilizer being studied. The specified morphotype (marked as "a" in Table 1) is characterized by the formation of large, rounded, matte milk-coloured colonies with uneven edges on MPA. The profile of the colonies is slightly cone-shaped, and the consistency is paste-like. Microscopy revealed medium-sized rods (0.9–1.0 × 1.5–3.0 µm) capable of endospore formation. The share of colonies of this bacterial morphotype ranges from 70% to 94% on granular fertilizers. During pasteurization of washes, the number of colonies of the specified morphotype decreased, but, in general, their share in the total number of microorganisms increased.

The next morphotype "b" is similar to the previous one, and is represented by rods, capable of sporulation, but the bacterial cells are slightly smaller in size (0.8–0.9 x 1.5–2.0 µm) and form white colonies on MPA. The number of representatives of this morphotype is, on average, 10–15 times smaller than the number of bacteria of morphotype "a".

In addition to the morphotypes described above, there are small amounts of cocci (morphotype "c"), which form yellowish colonies, as well as morphotype "d", represented by large rods (1.0–1.1 x 3.0–4.0 µm), connected in chains, that form white colonies on MPA. Pasteurization significantly reduces the number of these bacteria.

During the study, there were isolated and identified 112 bacterial cultures. Bacteria we assigned to the most numerous type "a" are representatives of *Bacillus amyloliquefaciens*; bacteria of the "b" morphotype are represented by *B. amyloliquefaciens* subsp. *plantarum*; the third morphotype is attributed to *Micrococcus* sp.; bacteria of morphotype "d" were identified as *B. cereus*.

Studies of the physiological activity of isolated microorganisms show that none of them are capable of nitrogenase activity. Among all the isolates studied, 20% showed growth-stimulating activity on barley sprouts, 28% showed phosphate-solubilizing activity, and some bacteria (36%) had antagonistic activity against pathogens of fungal diseases.

For further research, three strains were selected that may be agronomically promising – *B. amyloliquefaciens* B-22 (deposited in the depository of the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine under number IMB-B-8120), *B. cereus* 3/7 (deposited under number IMB-B-8119), *B. amyloliquefaciens* subsp. *plantarum* 5/13 (kept in the collection of the Institute of Agricultural Microbiology and Agroindustrial Manufacture of the National Academy of Agrarian Sciences under the number ICГМ-5/13). All three strains dissolved phosphorus mineral compounds and exhibited various degrees of cellulose-hydrolysing activity (Table 2).

Table 1. Number of microorganisms of various morphotypes on the mineral granular fertilizers (MPA)

Fertilizers	Without pasteurization			With pasteurization		
	Total number, CFU/g	Number of morphologically different microorganisms, CFU/g	% of the total number of microorganisms	Total number, CFU/g	Number of morphologically different microorganisms, CFU/g	% of the total number of microorganisms
Azofoska	42,900 ± 2973	[a]33,700 ± 2420	78.6	19,002 ± 1150	[a]19,000 ± 1149	99.9
		[b]5,500 ± 376	12.8		[b]1.7 ± 0.5	0.01
		[c]2,700 ± 120	6.3		[c]0.3 ± 0	0.001
		[d]1,000 ± 57	2.3		-	-
Polifoska	8300 ± 655	[a]7,000 ± 590	84.3	4,600 ± 347	[a]4,300 ± 315	93.5
		[b]300 ± 21	3.6		-	-
		[c]1,000 ± 44	12.1		[c]300 ± 32	6.5
Ammonium sulphate	77,100 ± 3468	[a]74,700 ± 3260	96.9	41,300 ± 2661	[a]39,700 ± 2500	96.1
		[b]1,700 ± 150	2.2		[b]1,300 ± 140	3.2
		[c]700 ± 150	0.9		[c]300 ± 21	0.7
Magnesium sulphate	8000 ± 731	[a]6,300 ± 594	78.8	41,300 ± 2661	[a]5,700 ± 405	95.0
		[b]1,000 ± 82	12.5		-	-
		[d]700 ± 55	8.7		[d]300 ± 45	5.0
Urea	17,000 ± 1413	[a]16,000 ± 1320	94.1	2,600 ± 212	[a]2,600 ± 212	100.0
		[c]1,000 ± 82	5.9		-	-
Kaliimag	13,000 ± 1098	[a]11,000 ± 911	84.6	6,900 ± 553	[a]6,600 ± 514	94.3
		[c]2000 ± 187	15.4		[c]300 ± 39	5.7
Simple superphosphate	12,600 ± 965	[a]11,300 ± 890	89.7	11,900 ± 1027	[a]10,600 ± 916	89.1
		[c]1,300 ± 75	10.3		[c]1,300 ± 111	10.9

Notes: Values are presented as mean ± standard error (n = 3).
a –milky, matte, large colonies with uneven edges and a “centre”, the shape of the colonies is concentric, the profile of the colonies is slightly cone-shaped, the consistency is paste-like; under the microscope, the rods are medium and large.
b – colonies similar to “a”, but whiter; under the microscope, the rods are slightly smaller in size than “a”.
c – yellow colonies (cocci under the microscope).
d – white colonies without a centre (segmented rods under the microscope) (see explanation in the text).

Table 2. Physiological activity of isolates from mineral granular fertilizers

Bacteria	Phosphate-mobilizing activity (phosphate dissolution zone, mm)	Cellulolytic activity of bacteria on CMC (translucency zone diameter, cm)
B. amyloliquefaciens B-22	1.0 ± 0.1	3.0 ± 0.4
B. cereus 3/7	2.0 ± 0.3	0.3 ± 0.1
B. amyloliquefaciens subsp. *plantarum* 5/13	0.9 ± 0.1	3.5 ± 0.3

Note: Values are presented as mean ± standard error (n = 5)

Furthermore, all three strains showed high growth-stimulating activity. Growth of sprouts of the test culture was registered even when the culture liquid was diluted 1 : 5000 (Table 3). According to the results of an *in vitro* laboratory study of antagonistic activity, it was established that *B. amyloliquefaciens* B-22, *B. amyloliquefaciens* subsp. *plantarum* 5/13 and *B. cereus* 3/7 showed an inhibitory effect on phytopathogenic micromycetes (Table 4). The strains *B. amyloliquefaciens* B-22 and *B. amyloliquefaciens* subsp. *plantarum* 5/13 showed the highest level of inhibition of mycelial growth of the micromycete *A. alternata*, the causative agent of alternariosis and the appearance of late dry spotting of potato tubers (Andriychuk, Skoreiko, 2023), the degree of inhibition was 84.4% and 97.2%, respectively.

Table 3. Growth-stimulating activity of bacteria

Variants	Barley root length, cm	Gain vs control, %
Control (H_2O)	1.9 ± 0.2	-
B. amyloliquefaciens B-22	2.8 ± 0.5	47.4
B. cereus 3/7	3.2 ± 0.5	68.0
B. amyloliquefaciens subsp. *plantarum* 5/13	2.5 ± 0.3	31.6

Notes: These are the results obtained by soaking barley seeds in bacterial suspensions diluted with water in a ratio of 1/5000. Values are presented as mean ± standard error (n = 10)

Table 4. Inhibitory activity of bacteria against phytopathogens, %

Phytopathogenic micromycetes	Strains of bacterial antagonists		
	B. amyloliquefaciens B-22	*B. amyloliquefaciens* subsp. *plantarum* 5/13	*B. cereus* 3/7
A. alternata 89	84.4 ± 3.2	97.2 ± 0.4	32.0 ± 2.0
F. culmorum 50716	68.5 ± 2.8	61.1 ± 1.4	37.0 ± 1.8
F. moniliforme 156	85.5 ± 2.2	51.8 ± 1.8	48.1 ± 1.8
F. oxysporum 311	62.9 ± 2.6	48.1 ± 1.8	50.4 ± 0.4
F. solani EK 1	64.4 ± 5.8	71.1 ± 2.2	25.3 ± 2.7
N. oryzae 3000	67.9 ± 1.9	60.4 ± 2.1	31.6 ± 0.4
N. oryzae 5000	83.7 ± 1.1	96.6 ± 1.1	27.7 ± 3.2

Note: Values are presented as mean ± standard error (n = 4)

In inhibiting the growth of fusariosis pathogens, *B. amyloliquefaciens* B-22 showed high activity, and *B. amyloliquefaciens* subsp. *plantarum* 5/13 and *B. cereus* 3/7 were active to a lesser extent. Significant suppression of the mycelium growth of the causative agents of fusariosis of growing potato plants – *F. oxysporum* and *F. solani* – was reported during their simultaneous cultivation with B-22 and 5/13. The highest antagonistic activity against the phytopathogen *N. oryzae* 5000 was found in *B. amyloliquefaciens* B-22 and *B. amyloliquefaciens* subsp. *plantarum* 5/13 – 83.7% and 96.6%, respectively. *B. cereus* 3/7 slightly inhibited the growth of phytopathogenic fungi of the genus *Nigrospora* (Table 4). Therefore, the studied representatives of the genus *Bacillus* show varying degrees of fungistatic effect to the tested phytopathogenic micromycetes.

According to the results of the observation, it was found that bacteria developed faster and, therefore, consumed nutrients more intensively than phytopathogenic micromycetes, which led to inhibition of their development. This allows to make assumptions about the competitive interaction between microorganisms and the formation of unfavourable conditions for the development of test cultures of micromycetes under co-cultivation with the studied bacteria, which is an example of passive (indirect) antagonism, in which some microorganisms suppress others.

The specified strains were used for the enrichment of mineral fertilizer (azofoska). The number of bacterial cells on fertilizer granules was within 2.5–3.0 × 10^7 CFU/g. The effectiveness of modified fertilizers was tested when growing potatoes in field conditions.

In the potato flowering phase, the content of chlorophylls a and b in the potato leaves was measured. The results of the research are presented in Table 5. Data show that *B. amyloliquefaciens* B-22-enriched azofoska provided an increase in the content of chlorophyll a to the greatest extent, and an increase in the content of chlorophyll b was predominantly provided by *B. amyloliquefaciens* subsp. *plantarum* 5/13-enriched fertilizer. The total value of the content of both chlorophylls was also the highest in the variant with *B. amyloliquefaciens* subsp. *plantarum* 5/13. The lowest indicators of chlorophyll content were registered in the control variant.

In addition to studying chlorophyll content in the leaves, the area of the leaf apparatus of potato plants was also measured. The results showed that the use of biologically modified azofoska for potato fertilization, regardless of the used bacterial strain, contributed to the improved indicators (Table 5).

The effect of biologically modified azofoska on the mass of the root system of potato plants was also assessed. Data in Table 5 show that except for the fertilizer enriched with *B. amyloliquefaciens* subsp. *plantarum* 5/13, other variants of biological modification had a positive effect on the formation of the mass of the root system of potato plants.

Table 5. Effect of biologically modified fertilizers on the formation of photosynthetic apparatus of potato plants and mass of roots

Variants of experiment	Leaf apparatus area, m^2/ha	Chlorophyll content, g/100 g			Mass of dry roots, g/plant
		a	*b*	*a* + *b*	
Non-enriched fertilizer, control	7,450 ± 752	75.21 ± 0.52	43.57 ± 1.15	118.78 ± 0.27	4.01 ± 0.53
B. amyloliquefaciens B-22-enriched fertilizer	14,953 ± 1948	76.03 ± 0.35	57.32 ± 1.20	133.35 ± 1.83	5.73 ± 0.44
B. amyloliquefaciens subsp. *plantarum* 5/13-enriched fertilizer	11,776 ±1687	73.17 ± 0.35	71.98 ± 4.41	145.15 ± 4.08	3.98 ± 0.75
B. cereus 3/7-enriched fertilizer	9,651 ± 974	73.49 ± 1.43	59.63 ± 3.08	133.12 ± 4.50	8.84 ± 0.95

Note: Values are presented as mean ± standard error (n = 4)

The calculation of the total yield of potatoes under the influence of biologically modified mineral fertilizer shows the positive effect of *B. cereus* 3/7-enriched azofoska – an increase in indicators by 28.7% was registered (Table 6). A significant increase in productivity was also obtained in the variant with *B. amyloliquefaciens* B-22 – by 21.9%. When using *B. amyloliquefaciens* subsp. *plantarum* 5/13, no reliable growth of the indicator was noted.

It should be noted that the use of biologically modified azofoska for potato fertilization had a positive effect on the marketability of products. Thus, the increase in the mass of marketable tubers with enriched fertilizer is significantly higher when compared with indicators of the total yield of the crop (Table 6). The yield of commercial tubers under the influence of *B. cereus* 3/7 increased by 39.2% to the level of the control variant, and under the influence of *B. amyloliquefaciens* B-22, by 29.2%. The use of *B. amyloliquefaciens* subsp. *plantarum* 5/13-enriched fertilizer also provided a significant (although the smallest in the experiment) increase in the studied indicator.

Thus, the increase in the mass of marketable tubers when using enriched fertilizer is significantly higher compared to the growth rates of the total crop yield (Table 6).

When measuring the starch content in potato tubers, depending on the type of biological modification of the mineral fertilizer, a significant increase in indicators was registered only in the variant with *B. amyloliquefaciens* B-22-enriched azofoska. In all other variants, a tendency to increase the starch content in products was registered (Table 7).

Nitrate content in potato tubers was generally low but decreased with the use of all bacterial strains studied in the experiment (Table 7).

Table 6. Potato yield when treated with biologically modified azofoska

Variants of experiment	Total yield, t/ha	Gain vs control		Yield of marketable tubers, t/ha	Gain vs control	
		t/ha	%		t/ha	%
Non-enriched fertilizer, control	22.3	-	-	19.3	-	-
B. amyloliquefaciens B-22-enriched fertilizer	27.2	4.9	21.9	24.9	5.6	29.2
B. amyloliquefaciens subsp. *plantarum* 5/13-enriched fertilizer	24.1	1.8	8.0	21.8	2.5	12.9
B. cereus 3/7-enriched fertilizer	28.7	6.4	28.7	26.9	7.6	39.4
HIP_{05}	2.4			1.9		

Note: Values are presented as mean ± standard error (n = 6)

Table 7. Effect of biologically modified fertilizers on the qualitative measures in potato tubers

Variants of the experiment	Starch content, %	Nitrate content*, mg/kg
Non-enriched fertilizer, control	11.1 ± 0.3	89.0 ± 4.9
B. amyloliquefaciens B-22-enriched fertilizer	12.0 ± 0.2	52.0 ± 3.2
B. amyloliquefaciens subsp. *plantarum* 5/13-enriched fertilizer	11.5 ± 0.1	30.5 ± 5.0
B. cereus 3/7-enriched fertilizer	11.5 ± 0.3	40.8 ± 3.5

Notes: Values are presented as mean ± standard error (n = 4).
* acceptable level of nitrates in potato tubers – 250 mg/kg.

DISCUSSION

The results showed that mineral granular fertilizers are contaminated with bacteria. At the same time, we did not detect micromycetes. Among the microorganisms on the surface of mineral granular fertilizers, regardless of its type, most belong to one morphotype, which is identified as *B. amyloliquefaciens*. A significant number of the studied bacterial group is represented by *B. amyloliquefaciens* subsp. *plantarum*. In addition to the mentioned types of bacteria, representatives of *B. cereus* and *Micrococcus* sp. are also found in small quantities.

In our opinion, the origin of bacteria contaminating granular fertilizers can be diverse: these are random microorganisms with which mineral fertilizers may come into contact during their production, transportation, storage, etc.; these are microorganisms that can contaminate the production mechanisms during the production of fertilizers and, thus, through a kind of aggressive "selection", inhabit the surface of the granules. Other ways of contamination are also possible. However, there one conclusion is beyond dispute: regardless of the type of fertilizers, representatives of one bacterial species – *B. amyloliquefaciens* – dominate on their surface. Therefore, representatives of this species are the most adapted to survive on granular fertilizers.

The study of the physiological activity of 112 isolates of bacterial cultures did not reveal diazotrophs among them. At the same time, certain isolates demonstrated such agronomically valuable properties as phosphate solubilization, growth stimulation, cellulose destruction and antagonism against certain pathogens of agricultural crops.

The use of microorganisms-enriched fertilizer when growing potatoes demonstrates the improvement of a number of indicators related to the production process of the culture. For example, the increase in the content of photosynthetic pigments in the leaves and the area of the photosynthetic apparatus of plants indirectly indicates the intensification of the photosynthesis process under these conditions. The growth and development of plants initiated by biologically enriched fertilizers is improved, which is largely related to photosynthesis. The increase in root mass can also depend on photosynthetic processes, at the same time it can be a consequence of the phytohormonal effect of enriched fertilizers.

As mentioned above, the studied bacteria showed indirect antagonism to certain micromycetes, the causative agents of plant diseases. However, other important mechanisms of active (direct) antagonism related to the action of antimicrobial compounds (peptides, lipopeptides, enzymes) and induction of systemic resistance in plants have been described for *Bacillus* (Huang et al., 2005; Choudhary, Johri, 2009; Gong et al., 2015; Shafi et al., 2017; Abbas et al., 2019). Furthermore, the indirect effect of bacteria on the resistance of plants to damage by phytopathogens by stimulating the growth and increasing the availability and level of absorption of nutrients is also possible (Idris et al., 2007). However, we did not notice any obvious signs of the manifestation of fungal diseases of potatoes, so it can be assumed that the positive effect of biologically enriched fertilizers on the formation of potato productivity was largely due to the action of phytohormonal compounds actively produced by the applied bacterial strains. This is indirectly confirmed by the indicators of root mass and growth of photosynthetic activity in experimental variants. Literature data indicate a positive effect of phytohormones on the development of the root system (Okushima et al., 2007; Ardakani and Mafakheri, 2011; Glick, 2012; Saidi and Hajibarat, 2021; Roychoudhry and Kepinski, 2022) and on the formation and activity of the photosynthetic apparatus of plants (Pilarska et al., 2016; Müller and Munné-Bosch, 2021).

The effect of the bacterial component on the dissolution of phosphates can also be quite real, since the bacteria used in the study have phosphate-solubilizing ability.

An important component of the mechanism of positive action of the bacteria used in the study may also be their production of hydrolytic enzymes. There is evidence in the literature that mineralization processes in the rhizosphere of plants may be enhanced due to the phytostimulatory effect of PGPM, with an increase in the intensity of photosynthesis and an increased supply of readily available carbon to the root zone, which contributes to the intensive development and activity of heterotrophic microorganisms in this niche (Oger et al., 2004; Blagodatskaya et al., 2010). Since the bacteria used to enrich mineral fertilizer produce hydrolytic enzymes (which we have shown on the example of cellulose hydrolyzing activity), it may be quite possible to intensify the mineral nutrition of plants using biologically enriched mineral fertilizer.

The criterion of the agronomic value of factors influencing the production process of agricultural crops is undoubtedly their effect on productivity. The increase in the yield of potatoes under the influence of certain variants of enriched mineral fertilizers indicates the significant potential of this method.

Microorganism-enriched azofoska influences the quality indicators of products. For example, *B. amyloliquefaciens* B-22 increases the content of ascorbic acid in tubers. At the same time, a decrease in nitrate content in tubers was registered in all experimental variants. This may suggest a certain metabolic "dilution" of nitrates (e.g., their transformation into amino acids). However, this conclusion needs analytical confirmation.

Therefore, the results of our research indicate the possibility of a significant impact on potato yield growth and product quality improvement without increasing mineral fertilizer doses. This is especially important for potatoes, as this crop requires high levels of nutrients in the soil to ensure constructive metabolism (Trawczyński, 2021; Chabani et al., 2025).

The results of our experiments require further research into the mechanisms of the influence of enriched fertilizers on the production process of potato and other agricultural crops. It is also necessary to study the terms of preservation of bacteria on granules, which will depend on the possibility of producing biologically enriched fertilizers at the relevant plants. It is also necessary to determine the possibility of enriching different types of mineral fertilizers, as well as the reaction of other types of crops to such fertilizers. It may be necessary to find other bacteria to enrich fertilizers, since the versatility of one strain or even a type of microorganism in terms of interaction with plants of different species is doubtful.

CONCLUSION

The surface of mineral granular fertilizers is contaminated with microorganisms, among which there are PGPB. Representatives of *Bacillus amyloliquefaciens* and *B. amyloliquefaciens* subsp. *plantarum* dominate on the surface of the granules. *B. cereus* and *Micrococcus* sp. are represented to a lesser extent.

The use of azofoska modified by applying suspensions of *B. cereus* 3/7 and *B. amyloliquefaciens* B-22 cells contributed to the increase in the content of chlorophylls a and b in the leaves of potato plants, area of the leaf photosynthetic apparatus, mass of roots, yield of the crop and improvement of the quality of the resulting products.

The prospects of enriching mineral fertilizers with agronomically valuable microorganisms for their use in the technologies of fertilizers production and cultivation of agricultural crops are positive.

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