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Synergistic interaction between *Azotobacter* and *Pseudomonas* bacteria in a growth-stimulating consortium

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Abstract:
Intensifying agricultural production involves an active use of agrochemicals, which results in disrupted ecological balance and poor product quality. To address this issue, we need to introduce biologized science-intensive technologies. Bacteria belonging to the genera *Azotobacter* and *Pseudomonas* have complex growth-stimulating properties and therefore can be used as a bioproduct to increase plant productivity. We aimed to create a growth-stimulating consortium based on the strains of the genera Azotobacter and Pseudomonas, as well as to select optimal cultivation parameters that provide the best synergistic effect. We studied strains *Azotobacter chroococcum* B-4148, *Azotobacter vinelandii* B-932, and *Pseudomonas chlororaphis* subsp. aurantiaca B-548, which were obtained from the National Bioresource Center "All-Russian Collection of Industrial Microorganisms" of Kurchatov Institute.

All the test strains solubilized phosphates and produced ACC deaminase. They synthesized 0.98–1.33 mg/mL of gibberellic acid and produced 37.95–49.55% of siderophores. Their nitrogen-fixing capacity ranged from 49.23 to 151.22 μg/mL. The strains had high antagonistic activity against phytopathogens. In particular, *A. chroococcum* B-4148 and *A. vinelandii* B-932 inhibited the growth of *Fusarium graminearum*, *Bipolaris sorokiniana*, and *Erwinia rhapontici*, while *P. chlororaphis* subsp. aurantiaca B-548 exhibited antagonism against *F. graminearum* and *B. sorokiniana*. Since all the test strains were biologically compatible, they were used to create several consortia. The greatest synergistic effect was achieved by Consortium No. 6 that contained the strains B-4148, B-932, and B-548 in a ratio of 1:3:1. The optimal nutrient medium for this consortium contained 25.0 g/L of Luria-Bertani medium, 8.0 g/L molasses, 0.1 g/L magnesium sulfate heptahydrate, and 0.01 g/L of aqueous manganese sulfate. The optimal cultivation temperature was 28°C.

The microbial consortium created in our study has high potential for application in agricultural practice. Further research will focus on its effect on the growth and development of plants, in particular cereal crops, under *in vitro* conditions and in field experiments.

Keywords: Biological preparations, sustainable agriculture, growth-stimulating microorganisms, microbial consortium, biocompatibility, phytohormones, siderophores

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INTRODUCTION

In the context of a growing population, food security is becoming a global issue, which calls for a significant increase in agricultural productivity [1–4]. Currently, high crop yields are achieved by using mineral fertilizers, pesticides, as well as chemical and synthetic growth stimulants [5]. They can cause serious harm to the environment, natural ecosystems, and human health [6, 7]. Residues from fertilizers and other chemicals contribute to air, water, and soil pollution [8]. Intensive use of fertilizers and pesticides leads to significant changes in the physical and chemical properties of the soil. These changes include its contamination with heavy metals and radionuclides, changes in pH, nutrient imbalance,

and soil compaction [9, 10]. Massah and Azadegan found that the long-term application of fertilizers based on nitrogen, phosphorus, and potassium compacted the soil layer [11]. This decreased its porosity, water permeability, and nutrient availability and therefore harmed the growth and development of wheat.

Moreover, the long-term supply of residual nitrogen, phosphorus, and sulfur compounds into the terrestrial ecosystem radically changes the composition and functions of its microbiota. Residues of nutrients in the soil can contribute to the extinction of some species and serve as a selective advantage for others [12]. For example, numerous studies have established a negative correlation between the number of diazotrophic microorganisms and intensive application of nitrogen fertilizers [13–15]. In the long term, this will significantly decrease the efficiency of biological nitrogen fixation and increase the soil's need for additional mineral fertilizers.

Chemical plant protection products have a negative impact on the diversity and number of soil microorganisms, as well as the enzymatic activity of soils [16]. For example, pesticide-treated soil showed decreased abundance and diversity of both fungal and bacterial communities. Moreover, the use of fungicides disturbed the processes of ammonification and nitrification [17]. In other studies, the fungicides benomyl, mancozeb, and tridemorph inhibited the soil enzymatic activity of dehydrogenase, urease, and phosphatase, while captan, trifloxystrobin, and thiram reduced the activity of phosphomonoesterase and urease [18–20].

The ability of agrochemicals to accumulate poses a particular danger. Vegetables, fruits, and grains growing in contaminated agricultural soils accumulate pesticides in their edible and inedible parts at concentrations that are high enough to cause serious health problems in animals and humans [21]. Although acute pesticide poisoning is now virtually unheard of, the long-term consequences of pesticide treatment remain a serious social problem. In particular, chronic toxicity caused by long-term exposure to low doses of pesticides can contribute to diseases such as cancer, asthma, dermatitis, endocrine disorders, reproductive dysfunctions, neurobehavioral disorders, and birth defects [22, 23]. Innovative technological solutions are needed to reduce the environmental load of modern agriculture, as well as ensure high-quality and safe production. Such technologies should aim at transitioning to more sustainable management of soil fertility [24]. Therefore, the development of biological preparations is currently on the rise [25, 26]. Rhizobacteria are a key component of such bioproducts. These microbial inoculants, or biofertilizers, can stimulate plant growth and increase the availability of nutrients. As a result, they reduce the use of chemical fertilizers and minimize their negative impact on the environment [27]. Modern research shows significant potential for the use of rhizobacteria as biological fertilizers for a wide range of agricultural crops [28]. The most important of them are those belonging to the genera *Azotobacter* and *Pseudomonas*.

Azotobacter is a genus of Gram-negative, non-symbiotic nitrogen-fixing aerobic soil bacteria, also known as azotobacteria. Having an oval or spherical shape, they can also form thick-walled cysts, which are dormant cells resistant to adverse environmental conditions. The genus includes about six species, some of which are motile due to the presence of peritrichous flagella, while others are nonmotile. Azotobacteria are known to use atmospheric nitrogen to synthesize cellular protein, which is mineralized in the soil, supplying nitrogen to crops. These bacteria are highly sensitive to environmental pH, high salt concentration, and temperature [29]. Therefore, these cultivation parameters need to be carefully selected to produce azotobacteria on an industrial scale.

Azotobacteria have a beneficial effect on the growth and productivity of agricultural crops. In particular, they synthesize bioactive and growth-stimulating substances, increase the microbial diversity of the rhizosphere, inhibit phytopathogens, improve the availability of nutrients, and enhance biological nitrogen fixation [30]. For example, *Azotobacter chroococcum* improves plant nutrition and increases soil fertility [31]. Other strains of the genus *Azotobacter* can produce amino acids when cultivated on a medium supplemented with various sources of carbon and nitrogen. Azotobacteria can also convert atmospheric nitrogen into ammonia to be absorbed by plants [32]. In addition, these bacteria are highly resistant to oxygen, which is especially important for nitrogen fixation in non-legume crops [33].

Nitrogen fixation plays a very important role in nitrogen homeostasis in the biosphere [34]. Biological nitrogen fixation also helps maintain soil fertility and increase crop yields. According to current research, azotobacteria annually fix about 20 kg of nitrogen per hectare. Therefore, they are successfully used in crop production as an alternative to mineral nitrogen fertilizers [35]. Crops treated with *Azotobacter* strains have been found to need smaller amounts of nitrogen fertilizers. For example, Felipe Romero-Perdomo *et al.* reported that the use of multiple *Azotobacter* strains nearly halved the need for nitrogen fertilizers [35]. Azotobacteria have a direct effect on plant growth by synthesizing plant growth hormones (e.g., auxins, gibberellins, and cytokinins). These hormones not only enhance plant growth and nutrient uptake, but also indirectly protect host plants from phytopathogens, as well as stimulate the development of other beneficial rhizosphere microorganisms [36, 37].

Azotobacteria can efficiently absorb iron from the environment by synthesizing siderophores, low-molecular-weight chelating agents with high affinity for Fe^{3+} ions [38]. Also, they can actively extract sparingly soluble iron salts from the environment by forming an iron-siderophore complex to be absorbed by membrane-bound receptors [39]. Since iron-siderophore complexes cannot be absorbed by other microorganisms, they give *Azotobacter* strains a competitive advantage. In addition, they protect plants from phytopathogens by limiting the availability of iron [39].

Since azotobacteria do not interact with plants symbiotically, they need to be used jointly with other microorganisms for maximum plant productivity. Numerous studies have shown that *Azotobacter* strains increase the activity of other growth-promoting microorganisms in the consortium, such as bacteria of the genus *Pseudomonas* [40, 41]. These rod-shaped, Gram-negative γ-proteobacteria with polar flagella have an extensive habitat [42]. Currently, the genus *Pseudomonas* includes over 100 species, many of which are widely used in biotechnology, biocontrol of phytopathogens, bioremediation, and plant growth stimulation [43, 44].

Pseudomonas bacteria are actively used as an inoculant for agricultural crops. Colonizing the surface and internal tissues of roots and stems, they are able to survive in various ecological niches thanks to highly developed adaptation mechanisms [44]. *Pseudomonas* bacteria promote plant growth by synthesizing ACC deaminase, increasing the availability of nutrients, and enhancing antioxidant activity [45]. Recent advances in the field of biofertilizers have led to the discovery of new strains with high phosphate-solubilizing activity. These include *Pseudomonas plecoglossicida*, a microorganism isolated from the rhizosphere of soybeans. This species can solubilize up to 75.39 mg/L of phosphate. In addition, it can accelerate plant growth by synthesizing an important plant phytohormone, indolyl-3-acetic acid (38.89 ppm) [46]. Another strain, *Pseudomonas* sp. PSB12, had the maximum phosphate solubilization index of 3.86 on Pikovskaya's agar medium. According to Weimin Chen *et al.*, this was mainly due to the synthesis of organic acids [47]. In another study, the presowing treatment of wheat seeds with phosphate-solubilizing and auxin-producing bacterium *Pseudomonas extremaustralis* IB-Ki-13-1A led to significantly higher yields [48].

In addition, *Pseudomonas* bacteria actively secrete phytohormones and volatile organic compounds [45, 49, 50]. Among them are auxins, phytohormones that stimulate cell division, elongation, and differentiation (particularly, indolyl-3-acetic acid) [51, 52]. *Pseudomonas mendocina* and *Pseudomonas alcaliphila* are auxin-synthesizing strains that stimulate seed germination and increase wheat yield, contributing to longer shoots, roots and ears, as well as higher seed weight [53]. In addition, the plants treated with the strains synthesizing indolyl-3-acetic acid show significant changes in the root system [54]. *Pseudomonas* bacteria also produce cytokinins, phytohormones that stimulate the division of plant cells and seed germination, activate the growth of dormant buds, and increase cell resistance to various unfavorable factors [55, 56]. *Pseudomonas* strains with complex phytohormonal activity include *Pseudomonas stutzeri* MTP40, *Pseudomonas putida* MTP50, and *P. putida* UKM B-398, which secrete indolyl-3-acetic acid, cytokinins, and gibberellins [57].

Pseudomonas species are widely studied to be used in biological control of phytopathogens. They synthesize various antimicrobial substances, including phenazine-1-carboxamide, amphicine, tensin, viscosine, massetolide, 2,4-diacetylphloroglucinol, pyrrolnitrin, pyoluteorin, and phenazine-1-carboxylic acid [43]. *Pseudomonas* strains are useful in controlling a number of diseases caused by fungal phytopathogens, including *Pythium* spp., *Fusarium solani*, *Rhizoctonia solani*, and *Phytophthora nicotianae* [58–61]. A partially purified siderophore obtained from the strain *Pseudomonas* JAS-25 completely inhibited the spores of *Fusarium oxysporum* f. sp. *ciceri*, *Fusarium udum*, and *Aspergillus niger*, which destroyed the mycelial hyphae of phytopathogens [62]. Similarly, hydrogen cyanide obtained from *Pseudomonas* strains exhibited bacteriostatic and antifungal effects against phytopathogenic fungi [63]. In particular, hydrogen cyanide derived from *Pseudomonas aeruginosa* (LES4) inhibited *Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomatoes [64]. In addition, *Pseudomonas* bacteria inhibit phytopathogens by competing for nutrients, inducing systemic resistance, producing siderophores, as well as synthesizing enzymes that destroy the cell wall (β-1,3-glucanase, chitinases, cellulases, proteases, etc.) [65]. For example, cyclolipopeptide orphamide induces systemic resistance in rice due to the expression of genes that protect it from the fungal phytopathogen *Cochliobolus miyabeanus* [66]. The siderophore pyoverdine produced by *P. putida* WCS358 induces systemic resistance in eucalyptus to prevent its bacterial wilt caused by *Ralstonia solanacearum* by [67].

Thus, the combined use of *Pseudomonas* and *Azotobacter* bacteria is an effective and environmentally friendly strategy for increasing productivity and sustainability of agricultural production. In addition, using consortia of growth-promoting microorganisms is more effective than using single strains. This is because bacteria naturally exist in taxonomically and metabolically diverse communities, rather than as monocultures of genetically identical strains [68, 69]. Genotypically diverse microbial communities are generally more resilient to various stresses than monocultures. They are also more competitive in the environment, which prevents the development of foreign strains. Further, polymicrobial communities distribute available resources more efficiently than individual genotypes [70]. As a result, they fill all the niches in the environment, preventing the invasion of extraneous microbiota [71]. Finally, diverse microbial communities are more resilient to changes in the abiotic environment, such as oxygen availability or pH levels, than isogenic cultures [72]. All these factors make consortia a promising strategy in agriculture.

There are two ways of creating microbial consortia: bottom-up and top-down. In the first approach, strains with specially selected functions are introduced into the nutrient medium to form an artificial microbial consortium. This approach has been proven to ensure mutually beneficial cooperation between the strains, with an evenly distributed load among them in the system [73]. The second approach is to isolate an already existing microbial consortium from the natural environment and ensure its functioning in a stable system. This method

has a significant drawback since natural microbial consortia have extremely complex compositions, with bacteria having both positive and negative effects on each other [74]. This can make their combined use quite difficult. Therefore, we chose the bottom-up approach for this study. In particular, we aimed to create a growth-stimulating consortium based on bacteria of the genera *Azotobacter* and *Pseudomonas*, as well as to select cultivation parameters that provide the best synergistic effect. The novelty of our research lies in our attempt to develop and optimize the biotechnological process of co-cultivating industrially significant strains available on the domestic market, as well as in the search for technological approaches to ensure maximum biological activity of the constructed consortia.

STUDY OBJECTS AND METHODS

The bacterial strains for this study were obtained from the National Bioresource Center "All-Russian Collection of Industrial Microorganisms" of Kurchatov Institute. They included:

– *Azotobacter chroococcum* B-4148 obtained from a spontaneous mutation of Rif-r and able to fix atmospheric nitrogen;
– *Azotobacter vinelandii* B-932 capable of fixing atmospheric nitrogen; and
– *Pseudomonas chlororaphis* subsp. *aurantiaca* B-548 isolated from *Zea mays*.

Analysis of cultural and morphological characteristics. To study the cultural characteristics of the strains, a low-concentration suspension of microorganisms was inoculated onto meat-peptone agar by the streaking method and cultivated for 18 h at 28 ± 2°C [75].

The morphological characteristics were examined using an AxioScope A1 upright microscope (Carl Zeiss, Germany) at a total magnification of 1000x. A fixed smear of microorganisms was stained using the Gram method [76].

Analysis of growth-stimulating properties and antagonistic activity. ***Solubilization of phosphates.*** The test strains were cultivated at 28 ± 2°C for 4 days on a medium containing (g/L) 5.0 calcium phosphate ($Ca_3(PO_4)_2$) (LenReaktiv, Russia), 20.0 glucose (Chem-ex, Russia), 0.2 sodium chloride (NaCl) (LenReaktiv, Russia), 0.1 magnesium sulfate heptahydrate ($MgSO_4 \times 7H_2O$) (Chem-Express, Russia), 0.01 aqueous manganese sulfate ($MnSO_4 \times H_2O$) (Khimplex, Russia), 0.01 iron II sulfate heptahydrate ($FeSO_4 \times 7H_2O$) (LenReaktiv, Russia), and 15.0 bacterial agar (Himmag, Russia). The growth of bacterial culture on Petri dishes indicated the strain's ability to solubilize phosphates [77].

Production of ACC deaminase. The test strains were cultivated on a medium containing (g/L) 2.0 ammonium sulfate ($(NH_4)_2SO_4$) (LenReaktiv, Russia), 4.0 monosubstituted potassium phosphate (KH_2PO_4) (ProfSnab, Russia), 6.0 sodium phosphate disubstituted dihydrate ($Na_2HPO_4 \times 2H_2O$) (Chem-ex, Russia), 0.2 magnesium sulfate heptahydrate, 0.001 iron II sulfate heptahydrate, and 15.0 bacterial agar. The medium also contained 1 mL of the following solutions: 16 μM boric acid (H_3BO_3) (LenReaktiv, Russia), 66 μM aqueous manganese sulfate, 433 μM zinc sulfate heptahydrate ($ZnSO_4 \times 7H_2O$) (LenReaktiv, Russia), and 313 μM copper sulfate pentahydrate ($CuSO_4 \times 5H_2O$) (LenReaktiv, Russia). The growth of bacterial culture on Petri dishes indicated the strain's ability to produce ACC deaminase [77].

Gibberellic acid. A bacterial suspension of the test strains was prepared on a Luria-Bertani liquid nutrient medium modified by Miller (LB) (Biolight, Russia) to a McFarland optical density of 0.8–1.0 using a Densichek plus densitometer (BioMerieux, France). After that, the suspension (1%) was added to the LB nutrient medium and cultivated in an LSI-3016R shaker-incubator (Daihan Labtech, South Korea) at 28 ± 2°C and 120 rpm for 24 h. The resulting culture liquid was centrifuged for 15 min at 7500 rpm. Then, 280 mL of a 1 M solution of zinc acetate dihydrate ($Zn(CH_3COO)_2 \times 2H_2O$) (ProfSnab, Russia) and a 10.6% solution of potassium ferrocyanide III ($K_3[Fe(CN)_6]$) (LenReaktiv, Russia) were added to 2 mL of the cell-free culture liquid, quickly mixed and centrifuged at 4500 rpm for 10 min. The resulting supernatant liquid was mixed with 30% hydrochloric acid (HCl) (LenReaktiv, Russia) in a 1:1 ratio and kept for 75 min at 22 ± 2°C. The optical density of the samples was determined in relation to 5% hydrochloric acid using a UV 1800 spectrophotometer (Shimadzu, Japan) at 254 nm. The synthesized gibberellic acid was quantified using a calibration graph of a standard solution of gibberellic acid (Diaem, Russia) in the range from 100 to 700 μg/mL [78].

Production of siderophores. For this, 1 mL of the cell-free culture liquid obtained as described above was mixed with 1 mL of freshly prepared Chorme Azurol S reagent (1.5 mL of a 0.016% solution of iron III chloride hexahydrate ($FeCl_3 \times 6H_2O$) (LenReaktiv, Russia) in a 10 M solution of hydrochloric acid was mixed with 7.5 mL of a 1.21% solution of chromasurol S (Chem-ex, Russia) and then distilled water was added to 100 mL). The resulting solution was kept for 20 min at 22 ± 2°C. The optical density was determined spectrophotometrically at a wavelength of 630 nm. Meanwhile, a control experiment was carried out under the same conditions, with a nutrient medium used as a control. The concentration of siderophores was determined using Eq. [79]:

$$C_{sid} = \frac{A_s - A_k}{A_v} \times 100$$

where C_{sid} is the concentration of siderophores, %; A_s is the optical density of the test sample; A_k is the optical density of the control sample.

Nitrogen fixation. A bacterial suspension was prepared as described above using a liquid nutrient medium containing (g/L) 20.0 sucrose (LenReaktiv, Russia), 5.0 magnesium sulfate heptahydrate, 1.0 potassium phosphate disubstituted trihydrate ($K_2HPO_4 \times 3H_2O$) (Chem-ex, Russia), 0.005 sodium molybdate dihydrate ($Na_2MoO_4 \times 2H_2O$) (LenReaktiv, Russia), 5.0 sodium chloride, 0.01 iron II sulfate heptahydrate, and 2.0 calcium

carbonate ($CaCO_3$) (LenReaktiv, Russia) [80]. The cultivation was carried out at 28 ± 2°C and 110 rpm for 48 h. The cells were separated from the culture liquid as described above. The amount of nitrogen in the cell-free culture liquid was determined using a Rapid N Cube nitrogen analyzer (Elementar, Germany) [81].

To determine the bacterial strains' ***antagonistic activity***, the phytopathogens *Fusarium graminearum* F-877, *Bipolaris sorokiniana* F-529, and *Erwinia rhapontici* B-9292 were obtained from the National Bioresource Center "All-Russian Collection of Industrial Microorganisms" of Kurchatov Institute. The phytopathogenic fungi and bacteria were cultivated in a test tube with potato-glucose agar and HMF agar, respectively. A daily culture of the test bacteria, which were grown on a liquid Luria-Bertani nutrient medium modified by Miller, was inoculated into Petri dishes on an agar medium using the deep method and incubated for 24 h at 28–30°C. Then, an agar block with the test culture was cut out and inserted into the well of an agar disk of another Petri dish with phytopathogens inoculated superficially using swabs from agar slants. The suspension with a McFarland turbidity of 0.8 (1.5×10^8 CFU/cm^3) was inoculated by the lawn method. The Petri dishes were refrigerated for 8 h at 4°C for the diffusion of metabolites of bacterial monocultures from the block into the agar with the test culture. Then, phytopathogenic fungi were incubated in a thermostat at 26–28°C [82].

Creating a microbial consortium. The ***biocompatibility*** of the test strains was analyzed by the well method. For this, bacterial suspensions were prepared as described above for gibberellic acid analysis. The test culture was applied with a Drigalski spatula to the surface of a Petri dish with LB agar medium using the spread plate method. Then, a 5-mm well was made for the cell-free culture liquid of the test culture to be cultivated for 24 h at 28 ± 2°C. The cultures were considered biocompatible if there was no inhibition of the test culture growth around the well [83].

Creating a consortium. For this, bacterial suspensions were prepared as described above for gibberellic acid analysis. Next, a certain number of microorganisms (depending on the composition and ratio) were applied to the sterile LB nutrient medium and cultivated under the conditions mentioned above. The supernatant was obtained by the previously described method.

The consortia were analyzed for their ability to produce ***gibberellic acid and siderophores***, as well as for their ***antagonistic activity*** against phytopathogenic microorganisms using the methods described earlier.

Selecting consortium cultivation conditions. The ***optimal temperature*** for consortium cultivation was determined using an RTS-8 plus personal multichannel bioreactor with non-invasive measurement of optical density in real time (Biosan, Latvia) at temperatures of 20, 24, 28, 32, 35, and 45°C. To cultivate a consortium, 3% of the inoculant composed of the bacterial suspensions in a certain ratio was added to the LB.

The ***nutrient medium base*** was selected from the following options:
– LB (control), 25 g/L;
– GMF broth, 30 g/L (Agat-Med, Russia);
– BTN broth, 30 g/L (Khimmedservis, Russia);
– tryptone-soy broth with yeast extract, 40 g/L (Germeon, Russia); and
– GRM broth, 20 g/L (Chem-ex, Russia)

The bacterial suspensions introduced into the consortium amounted to 3% of the nutrient medium. They were cultivated at the optimal temperature selected at the previous stage.

The ***carbon source*** was selected from the following options:
– Previously selected base (Control);
– Base + 4.0 g/L sucrose;
– Base + 4.0 g/L glucose;
– Base + 4.0 g/L molasses (Khimiya-express, Russia);
– Base + 8.0 g/L sucrose;
– Base + 8.0 g/L glucose; and
– Base + 8.0 g/L molasses.

The cultivation was carried out under the conditions described above.

The ***mineral component*** was selected from the following options:
– Control: selected base + selected carbon source;
– Medium No. 1: base + carbon source + 0.1 g/L magnesium sulfate heptahydrate;
– Medium No. 2: base + carbon source + 0.01 g/L aqueous manganese sulfate;
– Medium No. 3: base + carbon source + 0.1 g/L magnesium sulfate heptahydrate + 0.01 g/L aqueous manganese sulfate;
– Medium No. 4: base + carbon source + 0.2 g/L magnesium sulfate heptahydrate;
– Medium No. 5: base + carbon source + 0.02 g/L aqueous manganese sulfate; and
– Medium No. 6: base + carbon source + 0.2 g/L magnesium sulfate heptahydrate + 0.02 g/L aqueous manganese sulfate.

The cultivation was carried out under the conditions described above.

RESULTS AND DISCUSSION

Cultural and morphological characteristics of the strains. Figure 1 shows the cultural characteristics of the test strains. As can be seen, *Azotobacter chroococcum* B-4148, *Azotobacter vinelandii* B-932, and *Pseudomonas chlororaphis* subsp. *aurantiaca* formed 2–3, 3–4, and 1–2 mm round colonies, respectively. All the colonies had a smooth edge and a convex profile. Their color was beige, turning light brown as the culture aged.

Figure 2 shows the morphological characteristics of the test strains.

As can be seen, *A. chroococcum* B-4148 cells were Gram-negative coccobacilli, while the cells of *A. vinelandii* B-932 were Gram-negative, oval in shape, located singly or in pairs, and able to form cysts. The cells

of *P. chlororaphis* subsp. *aurantiaca* were Gram-negative and rod-shaped.

Growth-stimulating properties of the strains. The growth-stimulating activity of the test microorganisms is presented in Table 1.

As can be seen, all the test strains were able to solubilize phosphates and produce ACC deaminase. The amount of gibberellic acid they produced varied from 0.98 to 1.33 mg/mL, while the amount of siderophores ranged from 37.95 to 49.55%. The amount of nitrogen fixed by *A. chroococcum* B-4148, *A. vinelandii* B-932, and *P. chlororaphis* subsp. *aurantiaca* B-548 reached 151.22, 117.53, and 49.23 µg/mL, respectively.

Our results were consistent with those obtained by other scientists. For example, Biełło *et al.* found the ability for nitrogen fixation and phosphate solubilization in *A. chroococcum* NCIMB 8003 [84]. In the study by Alsalim, *A. chroococcum* demonstrated the ability to fix atmospheric nitrogen, solubilize phosphates, and synthesize siderophores and indolylacetic acid [85]. In another study, the isolates of *A. chroococcum* obtained from the rhizosphere of agricultural crops exhibited phosphate-solubilizing activity and produced indolylacetic acid, which changed the root architecture and increased the productivity of wheat [86]. *A. chroococcum* isolated from agricultural soils in China also showed the ability to synthesize indolylacetic acid and transform insoluble forms of phosphorus [87]. Kerečki *et al.* reported the ability of this species to produce ACC deaminase and synthesize indolyl-3-acetic acid [88]. However, the ability of *A. chroococcum* strains to produce gibberellins has been studied much less and is poorly covered in modern literature [89]. Therefore, our data expands the information about their growth-promoting mechanisms.

The growth-promoting activity of *A. vinelandii* has been confirmed by other modern studies. For example, *A. vinelandii* Khsrl isolated from the rhizosphere of *Chrysopogon aucheri* had the ability to synthesize a number of phytohormones, including indolyl-3-acetic, gibberellic, and abscisic acids [90]. McRose *et al.* reported the

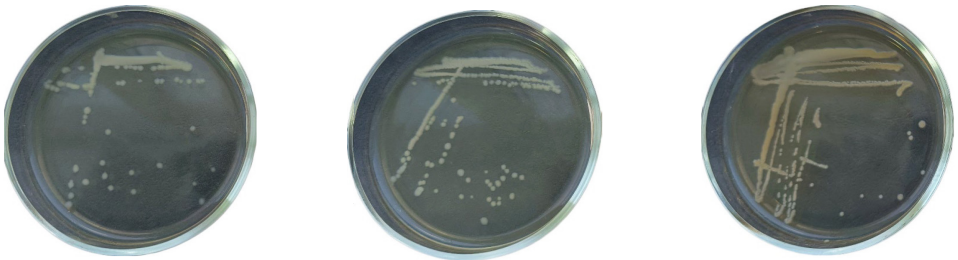

Figure 1 Cultural characteristics of the strains after 24 h of cultivation on meat-peptone agar (MPA): a – *Azotobacter chroococcum* B-4148; b – *Azotobacter vinelandii* B-932; c – *Pseudomonas chlororaphis* subsp. *aurantiaca* B-548

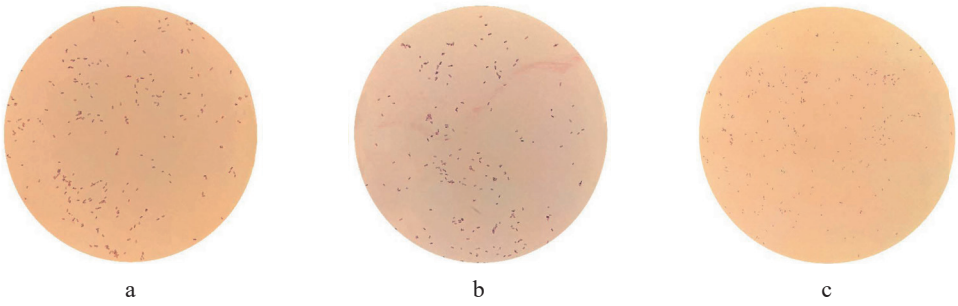

Figure 2 Morphological characteristics of the strains stained by the Gram method (×1000): a – *Azotobacter chroococcum* B-4148; b – *Azotobacter vinelandii* B-932; c – *Pseudomonas chlororaphis* subsp. *aurantiaca* B-548

Table 1 Growth-stimulating properties of the bacterial strains

Indicator	Strain		
	Azotobacter chroococcum B-4148	*Azotobacter vinelandii* B-932	*Pseudomonas chlororaphis* subsp. *Aurantiaca* B-548
Phosphate solubilization	+	+	+
ACC deaminase	+	+	+
Gibberellic acid, mg/mL	0.98 ± 0.03	1.30 ± 0.04	1.33 ± 0.04
Siderophores, %	47.77 ± 2.10	37.95 ± 1.53	49.55 ± 2.06
Nitrogen fixation, µg/mL	151.22 ± 6.23	160.64 ± 6.85	49.23 ± 2.13

strain's ability to produce siderophores under the conditions of iron deficiency [91]. They also noted that this ability reduced under the conditions of molybdenum and vanadium deficiency. Since these trace elements are part of nitrogenases, their deficiency may also limit the strain's nitrogen-fixing ability [92]. In another study, *A. vinelandii* AV7 isolated from the tomato rhizosphere produced indolylacetic acid and siderophores, as well as actively solubilized insoluble phosphates, increasing the plant's dry weight [93]. Shuvro *et al.* also reported this strain's ability to synthesize phytohormones and siderophores, as well as solubilize phosphates [94]. However, its ability to produce ACC deaminase has not been previously reported in literature, although our study showed the presence of this enzyme in the microorganism. Since ACC deaminase is involved in the metabolism of 1-aminocyclopropane-1-carboxylic acid (ACC), a precursor of ethylene in plants, it is an important factor in the plant's response to stress. Our data expands the understanding of the growth-promoting activity of *A. vinelandii*, since ethylene is a hormone that inhibits plant growth under abiotic stress. Thus, the strain's ACC deaminase activity may contribute to the plant's tolerance to unfavorable environmental factors.

The growth-promoting properties of *P. chlororaphis* subsp. *aurantiaca* were confirmed by a study of Rosas, who established the SR1 strain's ability to produce indolyl-3-acetic acid, hydrogen cyanide, and siderophores [95]. This strain has been reported to stimulate the growth of various crops such as alfalfa, wheat, soybeans, corn, and sugar cane, as well as to improve seed germination. Shi *et al.*, who studied another strain, SPS-41, reported its ability to produce indolyl-3-acetic acid and siderophores, as well as to solubilize phosphates [96].

Our next stage was to assess the antagonistic activity of the test bacteria against the most common fungal and bacterial phytopathogens, namely *Fusarium graminearum* F-877, *Bipolaris sorokiniana* F-529, and *Erwinia rhapontici* B-9292 (Fig. 3 and Table 2).

We found that all the studied strains exhibited antagonistic activity against the phytopathogens. *P. chlororaphis* subsp. *aurantiaca* B-548 had the greatest inhibitory

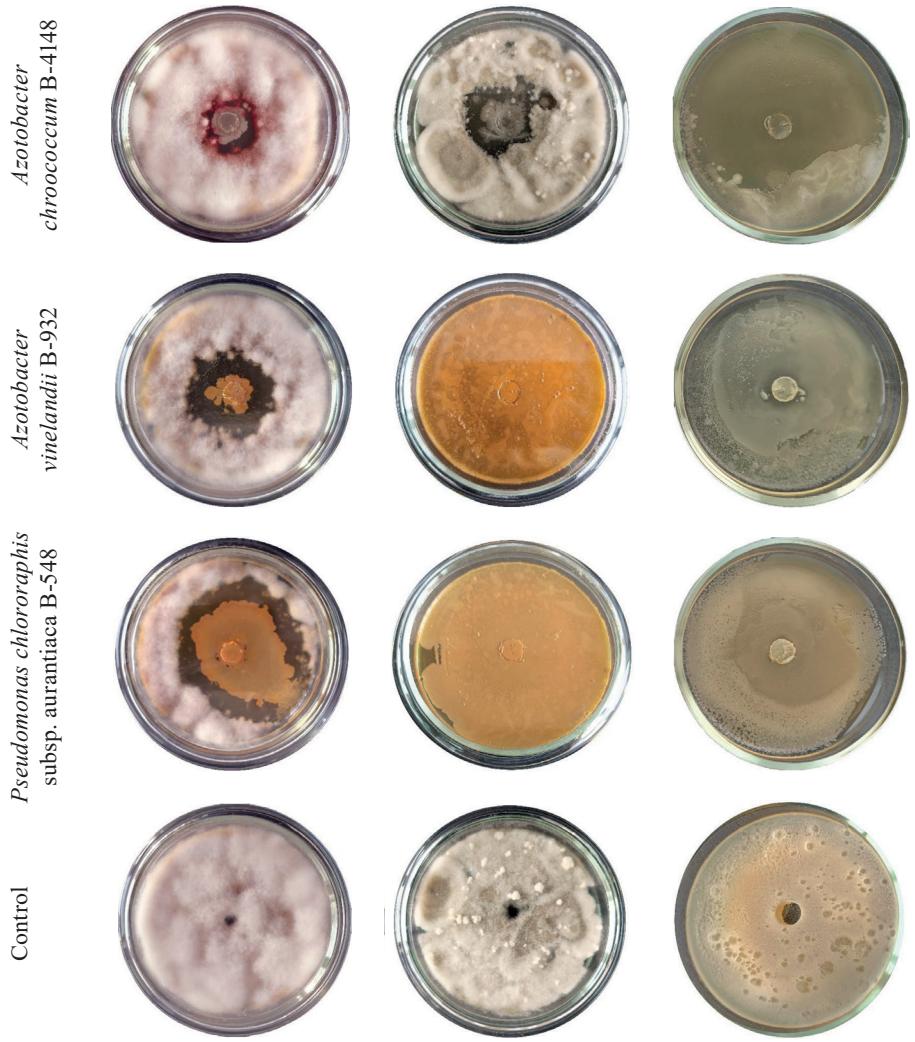

Figure 3 Antagonistic activity of the test strains: a – against *Fusarium graminearum* F-877, b – against *Bipolaris sorokiniana* F-529, c – against *Erwinia rhapontici* B-9292

Table 2 Antagonistic activity of the test bacterial strains

Strain	Phytopathogen inhibition zone, cm		
	Fusarium graminearum F-877	*Bipolaris sorokiniana* F-529	*Erwinia rhapontici* B-9292
Azotobacter chroococcum B-4148	2.55 ± 0.01	3.75 ± 0.02	7.50 ± 0.02
Azotobacter vinelandii B-932	4.25 ± 0.02	9.50 ± 0.03	2.00 ± 0.02
Pseudomonas chlororaphis subsp. *aurantiaca* B-548	6.25 ± 0.02	9.50 ± 0.03	–

Table 3 Consortia of the test strains

Consortium	Strain		
	Azotobacter chroococcum B-4148	*Azotobacter vinelandii* B-932	*Pseudomonas chlororaphis* subsp. *aurantiaca* B-548
No. 1	1	1	1
No. 2	2	1	1
No. 3	1	2	1
No. 4	1	1	2
No. 5	3	1	1
No. 6	1	3	1
No. 7	1	1	3

effect against *F. graminearum* (inhibition zone 6.25 cm) and *B. sorokiniana* (inhibition zone 9.50 cm). However, the strain showed no inhibitory effect against *E. rhapontici*. This may indicate mainly fungicidal properties of its metabolites. *A. vinelandii* showed the highest antagonistic activity against *B. sorokiniana* (inhibition zone 9.50 cm) but weak antibiotic properties against *E. rhapontici* (inhibition zone 2.50 cm). *A. chroococcum* exhibited the greatest inhibitory activity against this bacterial pathogen (inhibition zone 7.50 cm).

The strains' antibacterial and fungicidal properties can be confirmed by other modern studies. *A. chroococcum* is known to successfully suppress root rot [97]. In particular, Muslim *et al.* demonstrated the strain's ability to reduce the severity of tomato Fusarium wilt caused by the fungus *Fusarium solani* by more than 70% under greenhouse conditions [98]. Alsudani and Raheem also noted *A. chroococcum*'s ability to inhibit the growth of *F. solani*. The authors also reported its antagonistic activity against another phytopathogen, *Rhizoctonia solani* [99]. Pattaeva *et al.* reported that *A. chroococcum* strains N20, XH2018, and XU1 could synthesize a number of metabolites with fungicidal properties and suppress the growth of *Fusarium oxysporum* f. sp. *vasinfectum* [100].

The antagonistic properties of *A. vinelandii* have also been widely covered in modern literature. For example, Chuiko found that the soil isolate *A. vinelandii* IMV B-7076 exhibited antagonistic properties against a number of phytopathogens, including *Alternaria alternata* 16861, *Fusarium avenaceum* 50720, *Fusarium verticillioides* 50463, *Fusarium lactis* 50719, *Fusarium oxysporum* 54201, *Fusarium poae* 50704, and *F. solani* [101]. Bolaños-Dircio *et al.* also reported the strain's strong fungicidal properties. In particular, the authors showed that the cysts of this microorganism successfully suppressed the growth of *Fusarium brachygibbosum*, *Aspergillus niger*, and *Colletotrichum gloeosporioides* [102]. Since, there has been much less research into the antibacterial activity of *A. vinelandii*, our data on its suppression of *E. rhapontici* open up new possibilities for using the strain to control plant diseases.

Modern studies confirm the ability of *P. chlororaphis* subsp. *aurantiaca* to inhibit the growth of phytopathogenic microorganisms. For example, *Pseudomonas chromraphis* subsp. *aurantiaca soja* Q16 was reported to produce antibiotic substances that prevented the development of *F. oxysporum* and thus significantly improved potato growth [103]. Tagele *et al.* found that the KNU17Pc1 strain was capable of producing two types of phenazine derivatives and other antimicrobial substances. Due to this, the strain inhibited the growth of *Colletotrichum dematium*, *C. gloeosporioides*, *Fusarium oxysporum* f. sp. *melonis*, *Fusarium subglutinans*, and *Stemphylium lycopersici* [104]. *Pseudomonas chromraphis* subsp. *aureofaciens* M71, which was studied by Raio *et al.*, produced phenazine-1-carboxylic acid and successfully reduced the development of cancer caused by *Seiridium cardinale* in *Chamaecyparis pisifera* Endl [105]. Volatile organic compounds emitted by *P. chromraphis* subsp. *aureofaciens* SPS-41 successfully inhibited the growth of *Ceratocystis fimbriata* [106].

Creating microbial consortia. All the bacterial strains under study were biocompatible. Their ratios in the consortia are presented in Table 3.

We analyzed the ability of the bacterial consortia to produce gibberellic acid and siderophores (Table 4).

As can be seen, the amount of gibberellic acid varied from 1.23 to 1.44 mg/mL, while that of siderophores ranged from 48.21 to 54.46%. Consortia No. 1, 2, and 3 exhibited lower activity compared to the individual strains. Consortium No. 4 synthesized less gibberellic acid than the strains it was made of. Consortium No. 6 showed the greatest activity, producing 1.44 mg/mL of gibberellic acid and 54.46% of siderophores – 1.17 and 1.30 times as much, respectively, compared to the bacterial strains it contained.

Then, we analyzed the antagonistic activity of the consortia (Table 5).

As can be seen, Consortium No. 6 showed the greatest antagonistic activity against *F. graminearum* F-877 (inhibition zone 7.35 cm, averaging 4.31 cm for individual strains), *B. sorokiniana* F-529 (9.50 cm, averaging 8.35 cm for individual strains), and *E. rhapontici* B-9292 (8.10 cm, averaging 2.70 cm for individual strains). Consortium No. 1 had the lowest activity. Thus, based on

Table 4 The ability of bacterial consortia to produce gibberellic acid and siderophores

Consortium	Gibberellic acid, mg/mL		Siderophores, %	
	Amount	Average for strains	Amount	Average for strains
No. 1	1.23 ± 0.02	1.20	48.21 ± 1.33	45.09
No. 2	1.28 ± 0.02	1.15	48.66 ± 1.20	45.76
No. 3	1.39 ± 0.03	1.23	52.68 ± 1.75	43.31
No. 4	1.20 ± 0.02	1.24	50.45 ± 1.51	46.21
No. 5	1.34 ± 0.03	1.11	49.55 ± 1.49	46.16
No. 6	1.44 ± 0.03	1.24	54.46 ± 1.41	42.23
No. 7	1.32 ± 0.03	1.25	48.66 ± 1.30	46.87

Table 5 The antagonistic activity of bacterial consortia against phytopathogens

Consortium	Phytopathogens		
	Fusarium graminearum F-877	*Bipolaris sorokiniana* F-529	*Erwinia rhapontici* B-9292
No. 1	2.00 ± 0.01	2.20 ± 0.02	1.50 ± 0.01
No. 2	3.00 ± 0.02	5.30 ± 0.02	7.55 ± 0.03
No. 3	5.45 ± 0.02	8.05 ± 0.03	4.10 ± 0.02
No. 4	6.60 ± 0.03	6.75 ± 0.02	2.55 ± 0.01
No. 5	4.85 ± 0.02	3.60 ± 0.01	7.25 ± 0.02
No. 6	7.35 ± 0.03	9.50 ± 0.03	8.10 ± 0.03
No. 7	5.40 ± 0.03	9.50 ± 0.03	3.00 ± 0.02

the results, we selected Consortium No. 6 (*A. chroococcum* B-4148, *A. vinelandii* B-932, and *P. chlororaphis* subsp. *aurantiaca* B-548 in a ratio of 1:3:1) for further experiments.

Selecting cultivation parameters. Figure 4 presents the selection of the optimal cultivation temperature for Consortium No. 6.

At 20°C, the exponential phase on the LB nutrient medium began after 9 h of cultivation (optical density 0.33) and the stationary phase began after 22 h (optical density 2.70), with an optical density of 2.91 after 24 h. At 24°C, the exponential phase was observed after 6 h of cultivation (optical density 0.14) and the stationary phase, after 21 h (optical density 2.50), with an optical density of 2.67 after 24 h. At 28°C, the exponential phase started after 4 h of cultivation (optical density 0.10), with an optical density of 3.68 after 24 h. At 32°C, the exponential phase began after 4 h of cultivation (optical density 0.35), while the stationary phase began after 21 h (optical density 3.56), with an optical density of 3.63 after 24 h. At 35°C, the exponential phase was observed after 4 h of cultivation (optical density 0.46), while the stationary phase was noted after 18 h (optical density 2.67), with an optical density of 2.91 after 24 h. At 40°C, the exponential phase began after 4 h of cultivation (optical density 0.28) and the stationary phase, after 14 h (optical density 1.75), with an optical density of 2.02 after 24 h. Thus, the optimal cultivation temperature was 28°C.

Figure 5 and Table 6 show the selection of a nutrient medium base.

All the experiments were conducted at the optimal cultivation temperature of 28°C (Fig. 5). On the LB nutrient medium (control), the exponential phase began after 2 h of cultivation (optical density 0.93) and the stationary phase, after 21 h (optical density 4.01), with an optical density of 4.01 after 24 h. On the GMF broth, the beginning of the exponential phase was observed after 2 h of cultivation (optical density 0.48) and that of the stationary phase, after 23 h (optical density 6.65), with an optical density of 6.65 after 24 h. On the BTN broth, the exponential phase started after 2 h of cultivation (optical density 0.42), with an optical density of 4.32 after 24 h. On the tryptone-soy broth with yeast extract, the exponential phase was noted after 2 h of cultivation (optical density 0.46), with an optical density of 7.62 after 24 h. On the GRM broth, the exponential phase began after 2 h of cultivation (optical density 0.33), while the stationary phase, after 10 h (optical density 3.04), with an optical density of 3.26 after 24 h.

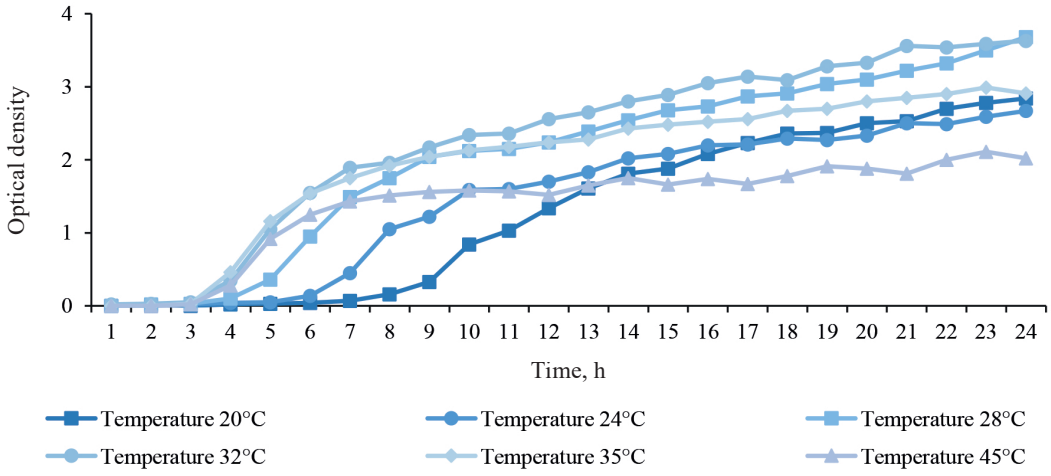

Figure 4 Optical density vs. cultivation temperature for Consortium No. 6

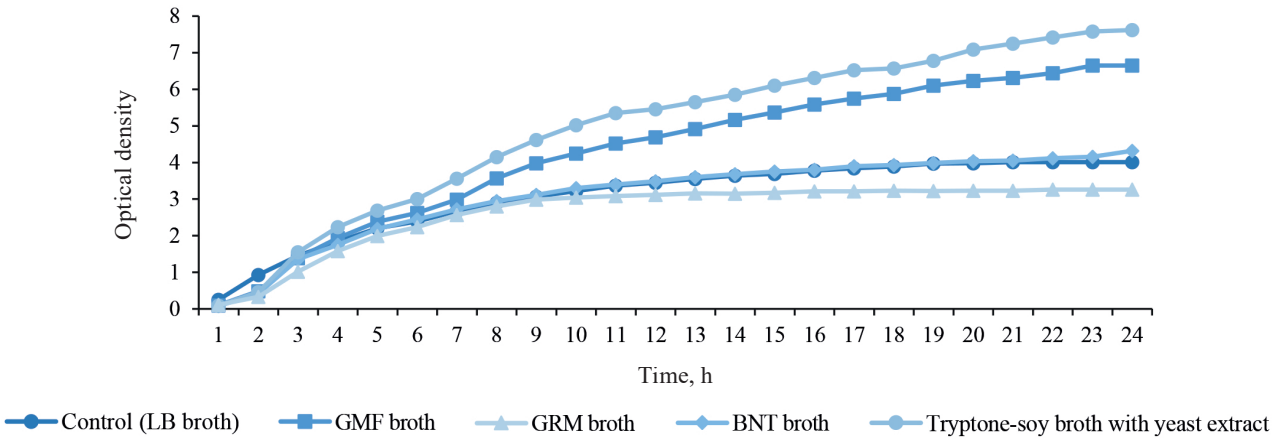

Figure 5 Optical density of Consortium No. 6 depending on the nutrient medium base

Table 6 The ability of bacterial consortia to produce gibberellic acid and siderophores depending on the nutrient medium base

Nutrient medium base	Gibberellic acid, mg/mL	Siderophores, %
LB (Control)	1.45 ± 0.06	58.04 ± 2.86
GMF broth	0.96 ± 0.05	17.86 ± 0.81
BTN broth	1.28 ± 0.06	55.36 ± 2.71
Tryptone-soy broth with yeast extract	1.16 ± 0.04	52.68 ± 2.43
GRM broth	0.91 ± 0.04	11.16 ± 0.58

Thus, Consortium No. 6 showed different growth rates and biomass values on different nutrient media. The tryptone-soy broth with yeast extract proved the best medium for bacterial growth, with the highest biomass values (optical density 7.62). The GMF broth also produced high biomass (optical density 6.65), but had a longer exponential growth phase. The GRM broth provided the least favorable conditions for growth, as evidenced by a shorter stationary phase and lower final biomass. However, growth-stimulating preparations require an optimized synthesis of growth-stimulating substances, which does not always correlate with biomass accumulation. Therefore, we carried out additional experiments to study the biochemical parameters of the consortium during its growth on various nutrient media.

As can be seen in Table 4, the amounts of gibberellic acid and siderophores varied from 0.91 to 1.45 mg/mL and from 48.21 to 54.46%, respectively. On the medium based on tryptone-soy broth with yeast extract, the consortium produced 1.16 mg/mL of gibberellic acid (0.29 mg less than on the control medium) and 52.68% of siderophores (5.36% less than on the control medium). Our results showed that the choice of a nutrient medium has a significant effect on both the growth and the biochemical activity of the consortium. The LB medium was considered optimal for cultivating the consortium. Despite the lower biomass observed on this medium, the consortium produced maximum amounts of gibberellic acid and siderophores, which is crucial for its targeted use.

Figure 6 and Table 7 show the selection of carbon source for the optimal composition of the nutrient medium.

All the experiments were carried out at the optimal cultivation temperature of 28°C (Fig. 6). On the LB medium (control), the exponential phase began after 2 h of cultivation (optical density 0.86) and the stationary phase began after 18 h (optical density 3.94), with an optical density of 4.09 after 24 h. On the LB + 4.0 g/L sucrose medium, the exponential phase started after 5 h of cultivation (optical density 0.59), while the stationary phase, after 22 h (optical density 4.00), with an optical density of 4.13 after 24 h. On the LB + 4.0 g/L glucose medium, the beginning of the exponential phase was observed after 5 h of cultivation (optical density 0.75), while that of the stationary phase, after 21 h (optical density 2.98), with an optical density of 3.04 after 24 h. On the LB + 4.0 g/L molasses medium, the exponential phase began after 5 h of cultivation (optical density 0.67) and the stationary phase, after 22 h (optical density 6.58), with an optical density of 6.75 after 24 h. On the LB + 8.0 g/L sucrose medium, the exponential phase started after 2 h of cultivation (optical density 0.75), while the stationary phase, after 22 h (optical density 4.18), with an optical density of 4.31 after 24 h. On the LB + 8.0 g/L glucose medium, the beginning of the exponential phase was observed after 5 h of cultivation (optical density 0.28), while that of the stationary phase, after 12 h (optical density 2.36), with an optical density of 2.66 after 24 h. On the LB + 8.0 g/L molasses medium, the exponential phase began after 5 h of cultivation (optical density 0.15) and the stationary phase started after 22 h (optical density 7.76), with an optical density of 7.63 after 24 h.

As can be seen in Table 5, the amounts of gibberellic acid and siderophores varied from 1.32 to 1.68 mg/mL and from 44.36 to 61.53%, respectively. The consortium exhibited its greatest activity on the medium containing LB + 8.0 g/L molasses (1.50 mg/mL of gibberellic acid and 61.53% of siderophores). Thus, molasses proved the most effective carbon source for cultivating this

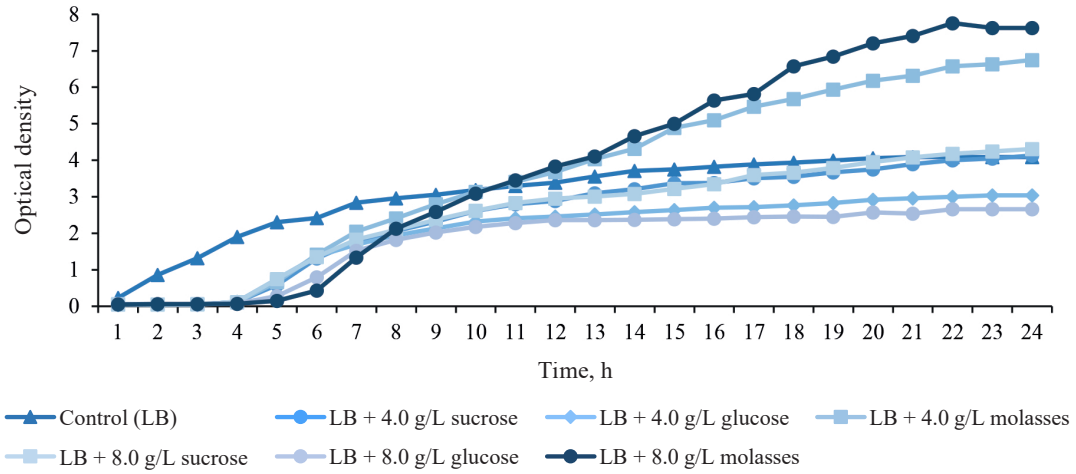

Figure 6 Optical density of Consortium No. 6 versus the composition of the nutrient medium

Table 7 The ability of bacterial consortia to produce gibberellic acid and siderophores depending on the carbon source

Nutrient medium	Gibberellic acid, µg/mL	Siderophores, %
LB (Control)	1.49 ± 0.06	56.24 ± 2.80
LB + 4.0 g/L sucrose	1.48 ± 0.05	49.86 ± 2.31
LB + 4.0 g/L glucose	1.32 ± 0.04	44.36 ± 2.05
LB + 4.0 g/L molasses	1.53 ± 0.08	59.40 ± 2.93
LB + 8.0 g/L sucrose	1.50 ± 0.06	51.23 ± 2.48
LB + 8.0 g/L glucose	1.39 ± 0.03	47.06 ± 2.20
LB + 8.0 g/L molasses	1.68 ± 0.04	61.53 ± 2.96

consortium, providing the greatest biomass and high production of growth-stimulating substances (gibberellic acid and siderophores). Therefore, molasses was chosen as the main source of carbon.

Figure 7 and Table 8 show the selection of mineral elements for the optimal composition of the nutrient medium.

According to Fig. 7, on the control medium, the exponential phase began after 6 h of cultivation (optical density 0.46) and the stationary phase, after 2 h (optical density 7.69), with an optical density of 7.69 after 24 h. On Medium No. 1, the exponential phase started after 6 h (optical density 0.51) and the stationary phase, after 21 h (optical density 7.53), with an optical density of 8.00 after 24 h. On Medium No. 2, the beginning of the exponential phase was noted after 4 h of cultivation (optical density 0.72), with an optical density of 7.93 after 24 h. On Medium No. 3, the exponential phase began after 5 h of cultivation (optical density 0.49), while the stationary phase, after 21 h (optical density 8.15), with an optical density of 8.31 after 24 h. On Medium No. 4, the exponential phase started after 6 h (optical density 0.31), while the stationary phase, after 21 h (optical density 4.91), with an optical density of 4.91 after 24 h. On Medium No. 5, the beginning of the exponential phase was observed after 5 h (optical density 0.26), while that of the stationary phase, after 17 h (optical density 5.02), with an optical density of 5.22 after 24 h. On Medium No. 6, the exponential phase began after 6 h of cultivation (optical density 0.21) and the stationary phase, after 15 h (optical density 3.59), with an optical density of 3.60 after 24 h.

As can be seen in Table 6, the amounts of gibberellic acid and siderophores varied from 1.20 to 1.79 mg/mL and from 52.28 to 65.56%, respectively. The consortium exhibited the lowest activity on Medium No. 6 (1.20 mg/mL of gibberellic acid and 52.28% of siderophores) and the highest activity on Medium No. 3 (1.79 mg/mL of gibberellic acid and 65.56% of siderophores).

Based on our results, the optimal composition of the nutrient medium was 25.0 g/L of LB, 8.0 g/L molasses, 0.1 g/L magnesium sulfate heptahydrate, and 0.01 g/L aqueous manganese sulfate. The optimal temperature was 28°C.

CONCLUSION

The bacteria belonging to the genera *Azotobacter* and *Pseudomonas* can be used in biopreparations to increase productivity and protect the plants from pathogens. These microorganisms have complex growth-stimulating properties and are also effective in microbial consortia.

In our study, all the test strains showed the ability to solubilize phosphates and produce ACC deaminase. Our data can clarify the growth-promoting effect of *Azotobacter vinelandii*, since its ability to produce ACC deaminase has been hardly covered in literature. ACC deaminase is involved in the metabolism of 1-aminocyclopropane-1-carboxylic acid (ACC), a precursor of ethylene in plants, which is an important factor in the plant's response to stress. Therefore, *A. vinelandii* can be used to increase plant productivity. The test strains synthesized an important phytohormone, gibberellic acid, in the range from 0.98 to 1.33 mg/mL and produced 37.95–49.55% of siderophores. All the strains showed a high ability to fix atmospheric nitrogen (49.23–151.22 µg/mL).

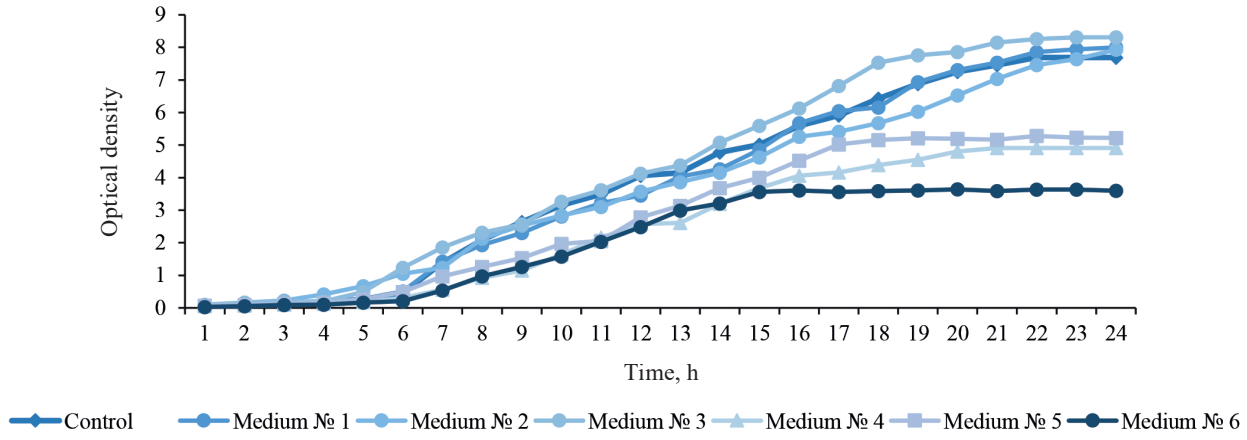

Figure 7 Correlation between the optical density of Consortium No. 6 and the composition of mineral salts in the nutrient medium

Table 8 The ability of bacterial consortia to produce gibberellic acid and siderophores depending on the mineral component in the media

Nutrient medium	Gibberellic acid, µg/mL	Siderophores, %
Control	1.66 ± 0.07	60.29 ± 2.76
Medium No. 1 (0.1 g/L magnesium sulfate heptahydrate)	1.70 ± 0.08	62.23 ± 3.06
Medium No. 2 (0.01 g/L aqueous manganese sulfate)	1.69 ± 0.08	61.54 ± 2.89
Medium No. 3 (0.1 g/L magnesium sulfate heptahydrate + 0.01 g/L aqueous manganese sulfate)	1.79 ± 0.05	65.56 ± 3.12
Medium No. 4 (0.2 g/L magnesium sulfate heptahydrate)	1.32 ± 0.04	53.61 ± 2.53
Medium No. 5 (0.02 g/L aqueous manganese sulfate)	1.46 ± 0.06	54.23 ± 2.49
Medium No. 6 (0.2 g/L magnesium sulfate heptahydrate + 0.02 g/L aqueous manganese sulfate)	1.20 ± 0.05	52.28 ± 2.44

The strains also exhibited antagonistic activity against common plant pathogens. In particular, *Azotobacter chroococcum* B-4148 and *A. vinelandii* B-932 inhibited the growth of *Fusarium graminearum*, *Bipolaris sorokiniana*, and *Erwinia rhapontici*. *Pseudomonas chlororaphis* subsp. *aurantiaca* B-548 exhibited antagonism against *F. graminearum* and *B. sorokiniana*.

Since all the test strains were biologically compatible, we created a number of microbial consortia from them. The greatest growth-stimulating activity was exhibited by Consortium No. 6 consisting of the strains *A. chroococcum* B-4148, *A. vinelandii* B-932, and *P. chlororaphis* subsp. *aurantiaca* B-548 in a ratio of 1:3:1. This consortium synthesized 1.44 mg/mL of gibberellic acid and 54.46% of siderophores, which was 1.17 and 1.30 times, respectively, as much as the constituent bacterial strains.

Next, we optimized the nutrient medium to ensure maximum efficiency. The LB medium was chosen as a base. Although the consortium had a low increase in biomass on this medium, it intensified the synthesis of gibberellic acid and siderophores (1.45 mg/mL and 58.04%, respectively). Molasses was chosen as the main source of carbon for the consortium since it provided the greatest increase in biomass, with the optical density of the culture liquid being 1.8 times as high as that of the control sample (without molasses). In addition, molasses contributed to high synthesis of target growth-stimulating substances. The greatest increase in biomass and synthesis of gibberellic acid and siderophores was observed when magnesium and manganese sulfates were added to the nutrient medium. Thus, the optimal composition of the nutrient medium included 25.0 g/L of LB, 8.0 g/L molasses, 0.1 g/L magnesium sulfate heptahydrate, and 0.01 g/L aqueous manganese sulfate. This composition provided the maximum yield of target metabolites. The optimal cultivation temperature for the consortium was 28°C.

Further studies of the created microbial consortium will involve its effect on plant growth and development both *in vitro* and in field experiments. Its effectiveness will be assessed in terms of plant biometric parameters, yield, as well as chlorophyll and nutrient contents. The resulting data will be used to develop a complex biopreparation that can replace chemical fertilizers and pesticides and ensure sustainable development of agriculture.

CONTRIBUTION

The authors were equally involved in writing the manuscript and are equally responsible for plagiarism.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding this publication.

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