Frontiers in Bioscience Elite

Front. Biosci. (Elite Ed) **2025**; 17(2): 33458
https://doi.org/10.31083/FBE33458

Original Research

Bacillus megaterium B-4801 Strain Efficiency in Growing Cereal Crops in Conditions Representative of Russia's Non-Chernozem Zone

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Academic Editor: Angela Roberta Lo Piero
Submitted: 3 December 2024 Revised: 24 January 2025 Accepted: 14 February 2025 Published: 9 April 2025

Abstract

Background: This study evaluates the possibility of using the experimental preparation "Naturost-M" based on the *Bacillus megaterium* B-4801 strain in crop production in conditions representative of Russia's non-Chernozem zone. The research objectives included whole genome sequencing of the B-4801 strain to determine its biotechnological potential and to study the effect of the preparation on the growth and grain productivity of several cereal crops. **Methods**: Whole genome sequencing of the *B. megaterium* B-4801 strain was performed at the Biotroph molecular genetic laboratory using the MiSeq platform (Illumina, Inc.). We conducted studies using cereal crops (barley, oats, and wheat) during the 2019–2022 growing seasons at the Vologda Research Center of the Russian Academy of Sciences experimental field. The preparation "Naturost-M" was applied twice: soaking seeds and spraying the phyllosphere of plants in the tillering phase. The raw and dry weights of experimental and control plants were measured in the tillering and earing phases during the growing season. We evaluated grain productivity at the end of the growing season. **Results**: Whole genome sequencing of the *B. megaterium* B-4801 strain revealed the main components of antimicrobial compound biosynthesis pathways, including a cluster of genes responsible for synthesizing enzymes for forming aliphatic unsaturated carboxylic acids containing 3–18 carbon atoms. Our research identified genetic loci encoding the synthesis of bacteriocins such as canosamine and polyketide ansamycin bacteriocins. The genome of the studied strain included clusters responsible for the biosynthesis of secondary metabolites such as siderophores and lantipeptides, as well as a whole range of genes responsible for various adaptation mechanisms of the strain to environmental conditions. Treatment of cereal crops with the experimental preparation "Naturost-M" contributed to an increase in growth parameters: raw weight was increased to 67% compared to the control, dry weight was up to 79% (depending on the year of study, phase of ontogenesis and culture), which occurred against the background of an increase in the content of photosynthetic pigments. Grain productivity grew in barley by 7–46%, oats by 12–31%, and wheat by 5–11% under conditions of small-plot experiments when using the preparation. **Conclusions**: The *B. megaterium* B-4801 strain has a certain biotechnological potential for crop production practice; experimental preparation created on its basis showed a stimulating effect on the growth and productivity of grain crops in conditions representative of Russia's non-Chernozem zone.

Keywords: *Bacillus megaterium*; whole genome sequencing; growth; grain productivity; photosynthetic pigments

1. Introduction

Maintaining food security is one of the most important tasks for any state and boils down to providing the entire population with food products in the required volume and quality. The main directions of state policy to ensure food security in the country undoubtedly include developing and maintaining the agro-industrial complex [1–3]. Cereals are of special interest; these are the most important resource for the food industry (providing the population with bread, cereals, etc.), as well as for fodder production and animal and poultry feeding (providing the population with milk, eggs, and meat) [4–7].

It is impossible for plants to reach their genetically programmed maximums without additional costs in the non-Chernozem zone, where low soil nutrition and complex variable weather conditions are limiting factors concerning crop cultivation [8]. Considering the modern trend toward the ecologization of various spheres of life and production, the use of biological preparations based on natural agents can be regarded as one of the ways to activate the growth and development of agricultural crops [8,9]. This trend can be observed in the increasing publication activity regarding biological agricultural inputs. For example, the PubMed portal found 52 publications on the query "biopreparations" from 1990 to 1999, 53 from 2000 to 2009, 91 from 2010 to 2019, and 111 from 2020 to 2024. In addition,

the Food and Agriculture Organization (FAO) of the United Nations has a long history of technical work on managing microorganisms and invertebrates for food and agriculture, including their use in integrated plant protection programs. For example, the FAO documents for the 14th session of the Commission include the thesis, "The prevention of the use of chemicals has a positive effect on microorganisms responsible for the nourishment of the soil, which is killed by applying pesticides and chemical fertilizers. Soil fauna and flora are encouraged, improving soil formation and structure and creating more stable systems". At the same time, FAO experts emphasize that pesticide residues are found in almost 40% of products used for food when using chemical plant protection products [10].

Biological preparations based on plant growth-promoting rhizobacteria (PGPR-group bacteria) can suppress the development of phytopathogenic organisms, stimulate plant growth through the production of enzymes, hormones, and siderophores, as well as through the mobilization of mineral nutrition elements [8,11–13]. In addition to the favorable effects listed for plant growth, microorganisms can induce changes in metabolic regulation in plants, such as cell wall biogenesis and transport of nutrients and ions [14,15].

Bacillus megaterium is a promising species of PGPR-group bacteria widely distributed in the soil from the point of view of its potential use in the crop industry [16]. For instance, a study by Dahmani *et al*. (2020) [17] showed a wide range of genomic features of the *B. megaterium* RmBm31 strain associated with the stimulation of plant growth; the research noted that the action of the strain increased biomass and positively changed the architecture of the root system of *Arabidopsis thaliana* seedlings both by physical contact of bacteria with roots and through the production of volatile organic compounds. The work of Huang *et al*. (2019) [18] demonstrates the ability of *B. megaterium* JX285 strain to solubilize inorganic phosphorus, which is attributed to the presence of genes associated with the production of organic acids that may be vital for phosphate conversion. Meanwhile, field studies by Romero-Munar and Aroca (2023) [19] presented a similar effect of *B. megaterium* bacteria on plant potassium availability. The *B. megaterium* MU2 strain was effective against drought, successfully colonizing wheat roots, increasing its biomass, relative water content, photosynthetic pigments, and osmolytes, and also revealed 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, indole-3-acetic acid (IAA) production and antagonistic activity against plant pathogens [20]. The study also proved that *B. megaterium* 501^{rif} reduces the phytotoxicity of herbicides by decomposing them in the rhizosphere of oats and maize while positively affecting plant growth [21]. Thus, the *B. megaterium* is of significant interest to agricultural science in terms of searching and studying promising strains, as well as developing effective biological preparations based on them that will meet the specified properties (biofungicide, plant growth stimulator, etc.) and work in the required conditions (climatic, soil, with specific varieties and crops, etc.).

B. megaterium B-4801 was previously used as a probiotic in farm animals, and its high antagonistic activity against several pathogens, including Salmonella, Pseudomonas, and Streptococcus, was demonstrated [22], which suggested an ability to biosynthesize a number of antimicrobial compounds such as organic acids and bacteriocins. In addition, we found the ability of the strain to degrade xenobiotics such as the herbicides glyphosate [23] and mycotoxins [24]. The mechanisms of biotransformation of compounds in different chemical natures are probably provided by the great lability of the strain's metabolism and the diversity of its enzyme systems. Therefore, the *B. megaterium B-4801* strain has high prospects of being applied in biocontrolling and growth-stimulating preparation. We have also demonstrated the ability of the strain to stimulate the growth of cereal crops and forage grasses [25,26].

This research aimed to determine the biotechnological potential of the *B. megaterium* B-4801 strain and evaluate the effect of the biopreparation created based on it on the growth and productive parameters of grain crops in Russia's non-Chernozem zone.

2. Materials and Methods

2.1 Location and Design of the Experiment

The test site was the experimental field of Vologda Research Center of the Russian Academy of Sciences. The test time was the growing season of 2019–2022.

2.2 Information about the Tested Biopreparation

Biopreparation "Naturost-M" based on live bacteria *B. megaterium* B-4801 strain was created by LLC Biotroph (Saint Petersburg, Russia). Bacteria were cultured on a nutrient medium, including beet molasses (2%) and mineral salts; sodium nitrate was a nitrogen source. The content of live bacteria from the original strain was at least 1×10^8 CFU in 1 mL of the preparation.

2.3 Whole Genome Sequencing of the Strain that Underlies Biopreparation

We carried out whole genome sequencing of the *B. megaterium* B-4801 strain of the preparation "Naturost-M" at the molecular genetic laboratory of Biotroph company (LLC Biotroph, Saint Petersburg, Russia). DNA was isolated according to standard methods using a Genomic DNA Purification kit (Thermo Fisher Scientific, Inc., Carlsbad, CA, USA).

DNA library for whole genome sequencing was constructed using the Nextera 819 XT kit (Illumina, Inc., San Diego, CA, USA). Nucleotide sequences were determined using a MiSeq instrument (Illumina, Inc., San Diego, CA, USA) with MiSeq Reagent kit v3 (300-cycle) (Illumina, Inc., San Diego, CA, USA). Invalid sequences and

Table 1. Weather conditions of the growing season in Vologda outskirts (according to the Weather and Climate portal, http://www.pogodaiklimat.ru).

Indicator	Norm*		2019		2020		2021		2022	
Average monthly temperature in May, °C	11.0	3.8**	12.0	2.7	9.0	15.2	12.1	5.4	8.0	8.1
Precipitation in May, mm	41.4		32.0		137.0		65.0		65.0	
Average monthly temperature in June, °C	14.5	4.1	16.1	3.2	16.0	3.8	19.1	1.6	16.0	3.8
Precipitation in June, mm	59.6		51.0		61.0		31.0		61.0	
Average monthly temperature in July, °C	17.9	3.7	14.1	11.3	17.0	8.4	19.1	1.4	19.2	4.2
Precipitation in July, mm	66.3		159.0		142.0		27.0		81.0	
Average monthly temperature in August, °C	15.2	4.6	12.1	7.6	14.1	5.0	16.0	8.7	19.3	1.4
Precipitation in August, mm	70.5		92.0		71.0		139.0		27.0	

Note: * The norm was calculated as an average value for 2000–2018; ** precipitation ratio to average monthly temperature.

adapters were removed using the Trimmomatic-0.38 program (https://www.osc.edu/book/ex-port/html/4385) [27]. Paired-end sequences filtered by length not less than 50 to 150 bp were assembled *de novo* (the SPAdes-3.11.1 genomic assembler, https://bioinf.spbau.ru/en/spades_for_remove) [28]. Functional genome annotation was performed using programs PROKKA 1.12 (https://github.com/tseemann/prokka) [29], RAST 2.0 (https://rast.nmpdr.org) [30] and antiSMASH (https://antismash.secondarymetabolites.org/#!/start) [31]. The KEGG Pathway database (http://www.genome.jp/kegg/) was used to assess the pool of genes associated with antimicrobial activity for constructing a metabolic map [32,33].

We also evaluated the content of various bioactive substances in the culture fluid of *B. megaterium* B-4801 strain by gas–liquid chromatography-QP2010 Plus (GCMS-QP2010 Plus, Kyoto, Japan) using Ultra-2 capillary column (25 m × 0.25 mm).

2.4 Objects of the Research

We used the following crops in the research: barley (*Hordeum vulgare* L.) of the variety Sonet, oat (*Avena sativa* L.) of the varieties Lev and Yakov, and common wheat (*Triticum aestivum* L.) of the variety Daria (the seed material was provided by the staff of the Department of Crop Production of the Northwest Scientific Research Institute of Dairy and Grassland Management named after A.S. Yemelyanov, Vologda, Russia). These varieties are acceptable for cultivation in the territory of Russia's non-Chernozem zone and, in particular, for the study area.

The seeds of experimental plants were soaked in working solution preparations for 2 hours, while the seeds of control plants were soaked in water. The concentration of the working solution was 1 mL of the preparation per 1 liter of water. In addition to inoculating seeds, single spraying of plants was also carried out after the emergence of the third leaf—the beginning of tillering—with working solutions of the same concentration, with a total preparation consumption of 1 L/ha.

2.5 Weather Conditions

Weather conditions in the 2019–2022 growing seasons differed significantly from each other and the calculated norm (Table 1). The beginning of the 2019 growing season was moderately wet and warm; increased precipitation and temperature decreases relative to the norm were observed in the second half of the period (July and August). The growing seasons of excessively wet and cool 2020 and hot and dry 2021 were less favorable for plant growth. The 2022 study period was generally in line with average annual trends, but the seed-sowing period (May) was cold and wet.

2.6 Assessing Growth and Productive Parameters of Plants in the Experimental Field

Seeds were sown in early May at depths of 3–5 cm using generally accepted seeding rates: 5.5 million germinated seeds per 1 ha. The accounting area was 2 m^2, and repetition was fourfold. The crops were tended manually in accordance with generally accepted agronomic practices. Mineral fertilizers, pesticides, and herbicides were not applied.

The soil in the experimental field is drained sod-podzolic, medium loamy. The chemical analysis results of the soil (FSBI State Center of Agrochemical Service "Vologodsky") show that the content of ammonia nitrogen was 4.2 ± 0.6 mg/kg, nitrate nitrogen was 38.9 ± 7.8 mg/kg, the mass fraction of mobile potassium was 261.0 ± 39.2 mg/kg, the mass fraction of mobile phosphorus was 260.0 ± 52.0 mg/kg, and the pH of the salt extract was 6.6 ± 0.1.

We considered biometric parameters of plants during the vegetation period (tillering stage, earing stage): raw and dry mass of the above-ground part of the shoot, number of leaves and shoots, and leaf surface area (n = 25). At the end of the vegetation, we formed sheaves, assessed grain productivity of crops (weight of total grain from 1 m^2) in laboratory conditions, and analyzed elements of yield structure: productive bushiness (n = 30), number of grains in inflorescence (n = 30), weight of 1000 grains (n = 5).

To estimate the dry weight of the above-ground part of the shoots, the biomaterial in the paper envelopes was

initially kept in a drying cabinet ShS-40 SPU (Smolensk SKTB SPU, Russia) at 90 °C for 30 minutes (to turn off the work of enzymes) and then at 60 °C until complete drying (to a constant mass value).

2.7 Assessing Photosynthetic Pigment Content in Leaves

We used the leaves of experimental and control plants to estimate the content of photosynthetic pigments. Pigments were agree extracted from plant leaves using 85% acetone [34]. The pigment content was determined using a PE-5400 spectrophotometer (Ecroskhim, Saint Petersburg, Russia), the pigments were extracted by triple extraction using 85% acetone, and the chlorophyll content was calculated using the Rebbelen equations (1).

$$\begin{aligned} &\text{C chl. } a = 10.3\ D_{663} - 0.918\ D_{644};\\ &\text{C chl. } b = 19.7\ D_{644} - 3.87\ D_{663};\\ &\text{C chl. } a + \text{chl. } b = 6.4\ D_{663} + 18.8\ D_{664}. \end{aligned} \quad (1)$$

D_{663} and D_{644} are the optical density of the solution at the corresponding wavelength. C chl. *a* and C chl. *b* are the corresponding concentrations of chlorophylls in the pigment extract.

2.8 Statistical Analysis

Statistical data processing was conducted according to standard methods using the Microsoft Excel 2019 Professional (Microsoft Corporation, Redmond, WA, USA). The tables present the average values of the indicators (M) and the respective standard deviation (± SD). The reliability of the difference in the sample means was evaluated using a confidence probability value of 0.95.

3. Results and Discussion

This study assessed the experimental preparation "Naturost-M", based on *B. megaterium* B-4801; the preparation has a fungicidal and antibacterial effect, and metabolites of living bacteria contribute to the accumulation of a vegetative mass of plants [22,25,26].

B. megaterium species are interesting for their physiological properties and ability to synthesize unusual and useful enzymes. Although *B. megaterium* species are usually called soil microorganisms, they are detected in various habitats, including seawater, bee honey, and dried foodstuffs. This bacteria type is widely used to stimulate the growth of multiple plants and bioremediate soils [35].

Using whole genome sequencing, we describe in detail the properties of the B-4801 strain of *B. megaterium* bacterium, which positively affects plant growth, development, and protection against pathogens. The genome of the studied *B. megaterium* B-4801 strain was annotated using the RAST toolkit (unique genomic identifier of the strain 1404.252). The genome of the strain is represented as a single ring chromosome of size 6,113,972 bp, which contains 37.5% guanine/cytosine (G/C) pairs. The chromosome composition is represented by 6324 open reading frames of polypeptide synthesis, particularly 129 of transport ribonucleic acid (tRNA) synthesis and six of ribosomal ribonucleic acid (rRNA) synthesis. The size of the plasmid part was 78,379 bp and included 23.5% G/C pairs.

Fig. 1 presents the distribution of the number of genes by functions in the metabolic systems of *B. megaterium* B-4801. Analysis of the strain genome sequencing results using the KEGG Pathway database allowed the main components in the pathways of antimicrobial compound biosynthesis to be defined. In particular, we identified a cluster of genes (*FabD*, *FabF*, *FabG*, *FabI*, *FabZ*, etc.) responsible for the synthesis of enzymes involved in the formation of aliphatic unsaturated carboxylic acids containing 3–18 carbon atoms (butyric, lauric, caproic, capric, caprylic, palmitic, myristic, stearic, and oleic acids) in the genome. This suggests a wide range of antimicrobial activity of the bacterial strain.

The analysis of genetic loci encoding the synthesis of antimicrobial metabolites, such as bacteriocins, in *B. megaterium* B-4801 reveals genes associated with the synthesis of canosamine, which belongs to the aminoglycosides group, and polyketide ansamycin bacteriocins, which belong to the macrolides group.

Clusters responsible for biosyntheses, such as secondary metabolites, including siderophores and lantipeptides, were detected in the genome of the *B. megaterium* B-4801 strain (Table 2). These metabolites are typical for strains of the genus *Bacillus* and have similarities in the organization of gene clusters. Wołejko *et al*. (2016) [36] posited that certain microorganisms can significantly contribute to the degradation of pesticides and other xenobiotics, positively affecting plant safety and soil fertility. For example, *B. mucilaginosus* bacilli can biodegrade pentachlorophenol, a toxic compound [37].

Secondary metabolites of siderophores, which are iron-chelating low molecular weight molecules that bind iron (Fe^{3+}), can form extracellular iron–siderophore complexes transported into the cytosol of bacteria [38]. Such siderophore-producing bacteria are often used to stimulate plant growth and development and to remediate the environment. It is known that siderophores can enhance microbial growth in natural or artificial environments and change the microbial community [39]. The formation of siderophores alongside the identified gene cluster (sequence length 15,889 bp) was performed using the direct participation of a protein from the IucA/IucC family with the involvement of genes in the complex of this cluster. The putative siderophore of the strain we studied is schizokinene, the synthesis process of which is similar to that in cyanobacteria of the genus Leptolyngbya PCC 7376 [40].

The lantipeptide (the sequence of the locus that participates in its synthesis is 23,141 bp) has 40% similarity to the cytolysin synthesis cluster ClyLl from plasmid pAD1, which belongs to the group of ribosomal-synthesized peptides.

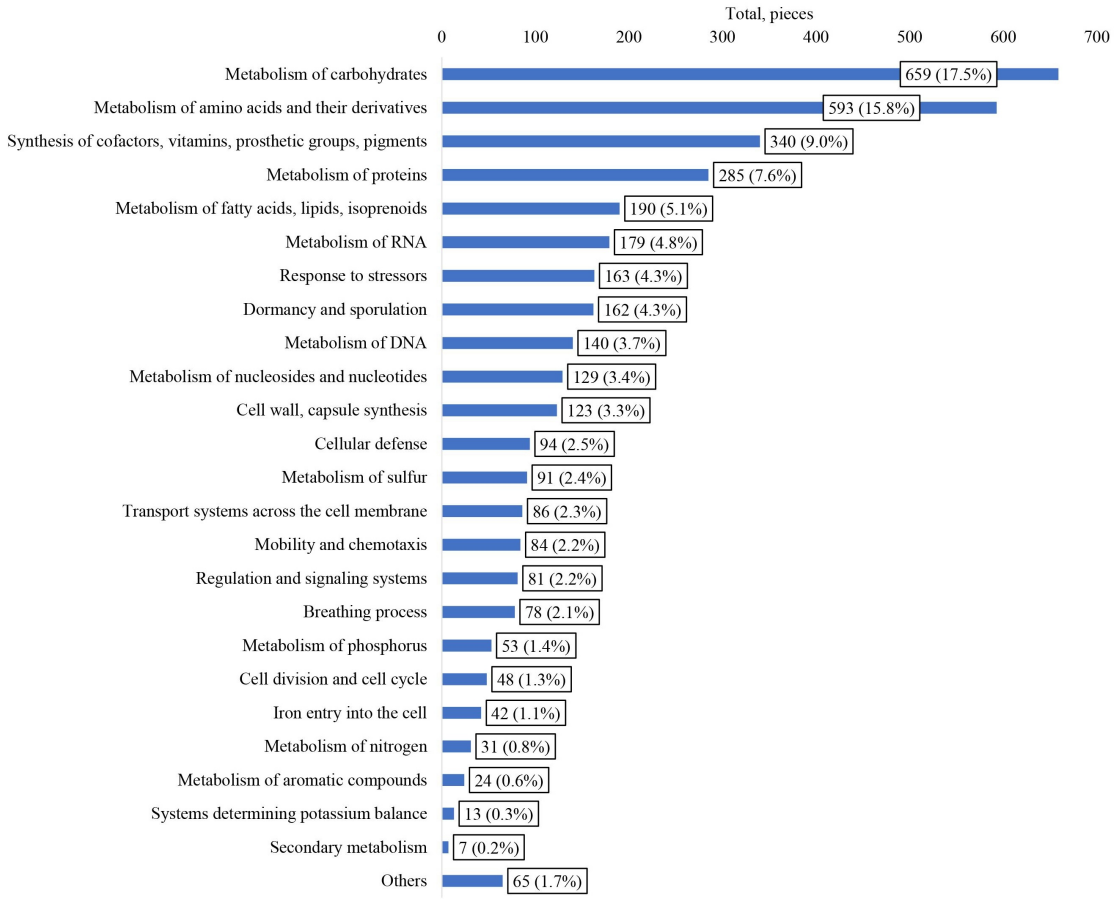

Fig. 1. Distribution of the number of genes by functions of metabolic systems of *B. megaterium* B-4801 bacteria. The column values represent the number of genes (units) agreeinvolved in the functions of specific metabolic systems; their proportion (%) is agree agreeprovided in parentheses.

Table 2. Characteristic of chromosome loci determining the biosynthesis of secondary metabolites of *B. megaterium* B-4801.

Locus size, bp	Chemical nature of the metabolite, (metabolite)	Similarity to bacterial homologous loci, %	
23,141	Lantipeptide (Cytolysin)	Cytolysin biosynthesis gene cluster ClyLl from plasmid pAD1	40
15,889	Siderophore (Schizokinen).	*Leptolyngbya* sp. PCC 7376	62

The genome of the *B. megaterium* B-4801 strain revealed a whole spectrum of genes responsible for various adaptation mechanisms (Table 3). For example, we have previously reported the increase in the yield of multiple crops against the background of joint application of *B. megaterium* and *Rhizophagus irregularis* in studies under arid climatic conditions and high-temperature stress [19].

Thus, as a result of studying the *B. megaterium* B-4801 genome, we revealed its unique features. We identified the genes associated with synthesizing the bacteriocin canosamine detected in the genome of the strain for the first time. Canosamine biosynthesis was previously detected in *B. cereus* [41], *B. pumilus* [42], and *B. subtilis* [43]. Synthesis of lantipeptides by *B. megaterium* strains has not been reported earlier either; there are only data on the synthesis of cytolysin A by bacteria *Escherichia coli* [44] and other bacteria in the Enterobacteriaceae family [45]. There are also data on the biosynthesis of other lantipeptides by *B. strainin*—a class I lanthipeptide biosynthetic gene cluster (lanBTC)—balucin, the action of which is directed against such food pathogens as *B. cereus* and *Listeria monocytogenes* [46]. Biosynthesis of siderophores has been previously identified in some bacillus strains; for instance, schizokinen, a citrate-containing dihydroxamate, is a siderophore produced by *B. megaterium* and *Anabaena* sp. [47,48].

We detected the presence of 28 volatile compounds according to the chromatographic analysis of the culture fluid of the *B. megaterium* B-4801 strain, which include aliphatic acids (six saturated C_2–C_6 and two unsaturated), three oxyacids, two phenyl-substituted acids, and two dicarboxylic acids. Regarding quantification, its major components are butyric acid and the corresponding hydroxy derivative, with relative contents of about 27% and 14%, re-

Table 3. Results of analysis of *B. megaterium* B-4801 genes responsible for various adaptation mechanisms.

№	Unfavorable external effects	Number of genes in the genome responsible for adaptation	Names of some genes responsible for adaptation
1	Oxidative stress	50	*Sod A, B, C, HPII, GloB*
2	Cold shock	18	*Csp A, D, G*
3	High osmotic pressure	17	*BetB, C, OpuA, B, ProU, ChA*
4	Heat shock	14	*RdgB, LepA, RsmE, HrcA, DnaK, GrpE*
5	Toxic stress	12	*CysA*
6	Carbon starvation	3	*RspB, CstA, CsrA*
7	Acid stress	11	*ArgR, RocF, D, A, R, E, SpeA, B, ArcA, D, F*

Table 4. Number of leaves of crops during vegetation under the action of the preparation based on the bacteria *B. megaterium* B-4801.

Response option		2019	2020	2021	2022
Tillering phase					
Hordeum vulgare Sonet	Control	5.1 ± 0.1	5.6 ± 0.2	no data	6.9 ± 0.1
	B. megaterium	6.4 ± 0.4*	5.6 ± 0.3	no data	7.5 ± 0.5
Avena sativa Yacov	Control	5.2 ± 0.4	no data	6.3 ± 0.3	5.6 ± 0.1
	B. megaterium	5.9 ± 0.2*	no data	7.6 ± 0.5	5.6 ± 0.3
Avena sativa Lev	Control	no data	5.3 ± 0.2	6.3 ± 0.3	6.7 ± 0.2
	B. megaterium	no data	5.3 ± 0.2	7.8 ± 0.7	6.8 ± 0.3
Triticum aestivum Dariya	Control	no data	7.4 ± 0.3	7.3 ± 0.5	6.1 ± 0.2
	B. megaterium	no data	9.4 ± 1.1	6.6 ± 0.6	7.2 ± 0.6*
Earing phase					
Hordeum vulgare Sonet	Control	12.1 ± 1.1	8.0 ± 0.5	no data	6.7 ± 0.1
	B. megaterium	15.1 ± 1.5*	8.0 ± 0.4	no data	7.7 ± 0.1*
Avena sativa Yacov	Control	14.9 ± 1.2	no data	7.8 ± 0.3	5.5 ± 0.1
	B. megaterium	16.0 ± 1.8	no data	11.5 ± 1.3*	5.6 ± 0.1
Avena sativa Lev	Control	no data	8.1 ± 1.1	6.1 ± 0.5	4.9 ± 0.2
	B. megaterium	no data	9.8 ± 0.6*	8.1 ± 0.7*	5.9 ± 0.2*
Triticum aestivum Dariya	Control	no data	7.9 ± 0.9	6.4 ± 0.4	3.8 ± 0.1
	B. megaterium	no data	12.8 ± 1.3*	8.5 ± 0.9*	4.5 ± 0.1

Note: the differences are significant when comparing these groups *$p \leq 0.05$.

spectively. In addition, methyl butyric acid has been identified as 1.54%. Among other classes of organic compounds, it is worth noting the presence of a polyhydroxy compound, 2-methylpropantriol, with a relative content of 13.6%.

Moreover, experimental data show that a significant accumulation of glutamic acid (100.6 µg/mL) and aspartic acid (10.8 µg/mL) is observed in the culture fluid sample of *B. megaterium* B-4801. The literature presents the data proving that aspartic and glutamic acids play an important role in stimulating plant growth and increasing stress resistance. For example, it has been shown that inoculation of plants with PGPR-bacteria consortia improves nutrient supply from the soil and raises drought tolerance in barley and chickpeas. At the same time, aspartic acid produced by bacteria plays an important role [49,50]. Moreover, glutamic acid can act as an external signal to induce complex changes in root growth and development and also increases the thermotolerance of plants [51–53].

The data on detecting riboflavin (vitamin B_2) in the culture fluid of *B. megaterium* B-4801 in the amount of 1.7 µg/mL are particularly interesting. These data show that riboflavin increases the resistance of plants, which become less sensitive to fungi and parasites, and also increases the resistance of plants to abiotic stress conditions [50].

Thus, *B. megaterium* B-4801 has some biotechnological potential for use in crop production; further studies of its effect on crop growth and productive qualities are reasonable.

Based on the four-year results of the field study, we can note that the treatment of grain crops with the experimental preparation “Naturost-M”, in general, contributed to the increase in the growth parameters of plants. The stimulating effect of the biopreparation was insignificant at the initial stages of ontogenesis. For instance, the number of shoots in experimental and control variants differed irrelevantly, especially at the beginning of vegetation. The number of leaves in barley of the Sonet variety in the tillering

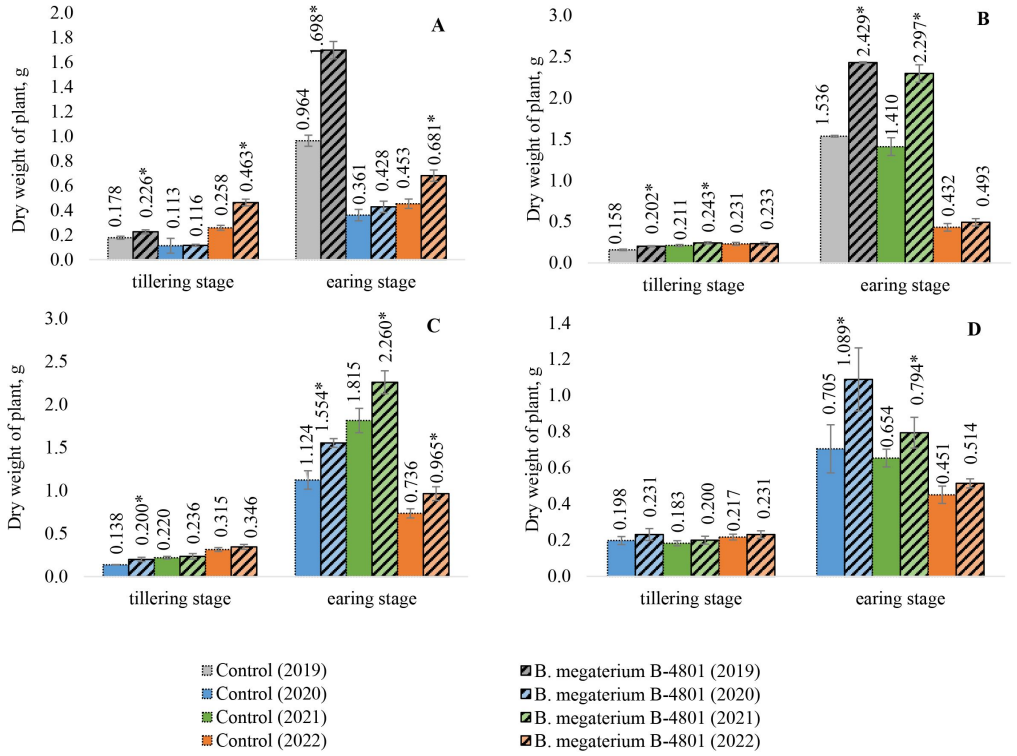

Fig. 2. Dry weight of crops during vegetation under the preparation action based on the bacteria *B. megaterium* B-4801. The objects of the research are (A) barley of the Sonet variety, (B) oats of the Yakov variety, (C) oats of the Lev variety, and (D) wheat of the Daria variety. The column values represent the mean, and the whiskers represent the minimum and maximum values of the data. The differences are significant when comparing these groups $*p \leq 0.05$.

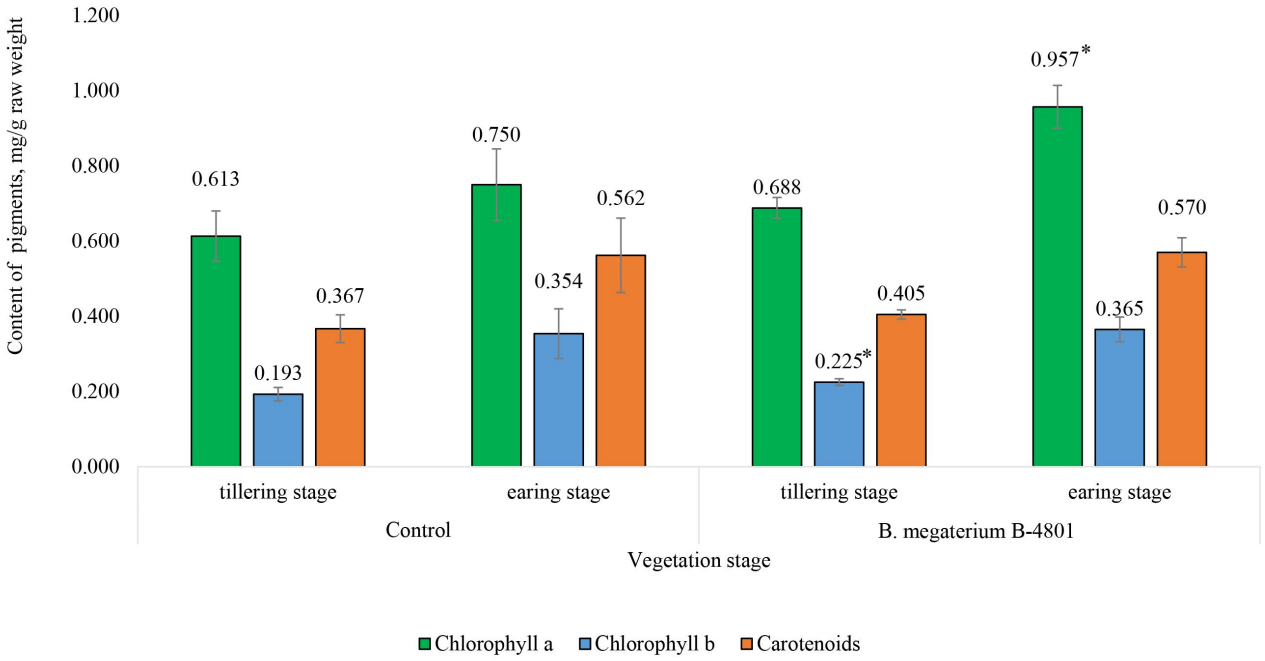

Fig. 3. Content of photosynthetic pigments in leaves of spring barley of the Sonet variety (2020 experiment). The column values represent the mean values, and the whiskers are the minimum and maximum values of the data. The differences are significant when comparing these groups $*p \leq 0.05$.

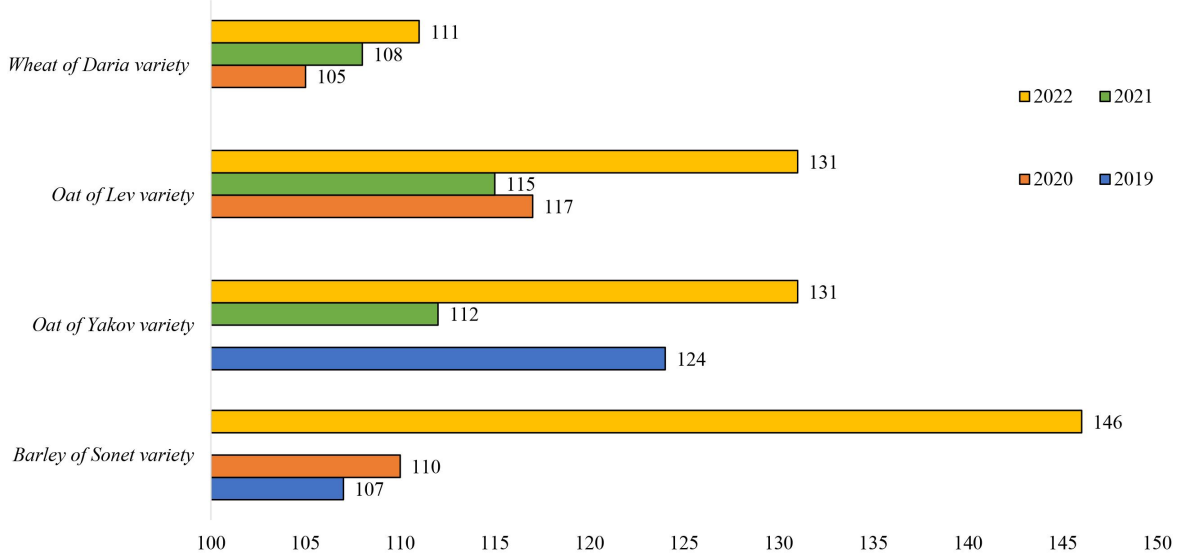

Fig. 4. Relative grain productivity of crops under the action of the preparation based on the bacteria *B. megaterium* B-4801 (% of control). The column values represent crop yield relative to the control (%).

Table 5. Grain productivity of crops under the action of the preparation based on the bacteria *B. megaterium* B-4801.

Response option		Grain productivity, centner/ha			
		2019	2020	2021	2022
Barley of the Sonet variety	Control	27.8 ± 1.4	20.9 ± 0.6	no data	18.0 ± 0.6
	B. megaterium	29.7 ± 1.2	22.9 ± 0.8*	no data	26.3 ± 1.6*
Oat of the Yakov variety	Control	25.1 ± 1.2	no data	17.7 ± 1.6	17.0 ± 1.0
	B. megaterium	31.3 ± 2.9*	no data	19.9 ± 1.0	22.3 ± 1.1*
Oat of the Lev variety	Control	no data	16.4 ± 1.3	12.8 ± 1.2	19.6 ± 0.2
	B. megaterium	no data	19.2 ± 1.2*	14.7 ± 0.7*	25.6 ± 0.2*
Wheat of the Daria variety	Control	no data	29.6 ± 2.2	28.3 ± 2.1	31.2 ± 2.0
	B. megaterium	no data	31.2 ± 2.7	30.5 ± 2.8	34.5 ± 2.9

Note: the differences are significant when comparing these groups *$p \leq 0.05$.

phase differed substantially only in the experiment of 2019 (25% difference), in oat of Yakov and Lev varieties, only in the experiment for 2021 (21% and 24%, respectively); meanwhile, differences in wheat of variety Daria in this indicator reached 10–27% depending on the year of study (Table 4).

One of the most important growth parameters is plant weight indices. Raw and dry weights in the experimental groups of tested crops usually exceeded the control values already in the tillering phase. For instance, in barley of the Sonet variety under the effect of preparation "Naturost-M", the differences in dry weight reached 3–79%; in oats of the Lev variety, it was 7–45%, 1–28% in oats of the Yakov variety, and 7–17% in wheat of the Daria variety (Fig. 1). It is worth noting that the values of dry mass in plants in the 2022 experiment were greater; probably, the growth of plants was more intensive at the beginning of vegetation in 2022.

The experimental preparation "Naturost-M" had a more pronounced effect on the weight parameters of test crops in the earing phase. For example, in the 2019 experiment, the crude weight of barley plants of the Sonet variety under the action of this preparation increased by 48%, while the dry weight increased by 76%; in the 2020 experiment, these metrics increased by 7% and 19%, respectively, while in the 2002 experiment, they increased by 47% and 50%, respectively. In studies using oats of the Yakov and Lev varieties, the observed trend was similarly repeated: raw weight in plants of experimental variants exceeded the control by 19–67% and 14–41%, while for the dry weight, it was by 14–63% and 25–38%, respectively. The effect of the preparation on wheat in the earing phase also increased the raw and dry mass of the plant; in the 2020 experiment, the difference reached 42–54%; in the 2021 experiment, it reached 1–21%; in the 2022 experiment, it was 14–17% (Fig. 2).

Dry matter accumulation is certainly related to photosynthetic productivity [54]. This is also consistent with the results of assessing the assimilative surface of the plant. For example, in the 2019 experiment, the area of the assimilation apparatus of the barley of the Sonet variety statistically reliably increased by 23% at the application of the

experimental preparation "Naturost-M". The average leaf area when using this microbial preparation in the flowering phase of the crop was raised by 34% relative to the control in the experiment using the wheat of the Daria variety. In addition to an increase in the assimilative apparatus area, barley tended to increase the content of photosynthetic pigments in leaves under the action of this preparation. For example, the preparation increased the content of chlorophyll *a* by 12–28%, chlorophyll *b* by 3–17%, the sum of chlorophylls by 15–36%, and carotenoids by 1–10% in the tillering phase of the 2020 experiment (Fig. 3). Similar results on the changes in photosynthetic pigment content were obtained in the Rashid *et al*. (2022) study [20], which studied the reaction of wheat to inoculation with bacteria *B. megaterium* MU2 and *B. licheniformis* MU8. The research shows that the bacteria *B. megaterium* MU2 promoted an increase in chlorophyll *a, b,* and carotenoid content by 59%.

The increasing area of the assimilative surface against the background of rising photosynthetic pigment content suggests that the energy availability in the experimental plants was higher than in the control. Notably, the most tangible differences in growth parameters of experimental and control variants were observed in more comfortable weather conditions in the growing seasons of 2019 and 2022. Excessively wet conditions in 2020, as well as dry and hot conditions in 2021, probably had a negative impact on the viability of bacteria.

Based on the obtained data on biometric indices of plants in different phases of vegetation, we can say that, in general, the bacteria *B. megaterium* B-4801 had a noticeable effect on the growth processes of the tested plants. Stimulation of plant growth by Bacillus bacteria probably occurs through their synthesis of biologically active substances, increasing phosphate dissolution and iron chelation. López-Bucio *et al*. (2007) [55] and Ortíz-Castro *et al*. (2008) [56] mentioned that the effect of *B. megaterium* on plants may be associated with the synthesis of cytokinins, which primarily leads to more active development of the root system of plants, especially lateral roots.

As a result, changes in growth parameters affected the economic productivity of crops. For instance, grain productivity of the barley of the Sonet variety under the action of the experimental biopreparation "Naturost-M" substantially increased in the 2022 experiment; the difference with the control reached 46%. Notably, such high differences in the 2022 experiment are associated with a significant gain in experimental plants and a noticeable lag in the control from the "normal" barley indicators in all vegetation phases. In the studies during 2019–2020, the grain productivity of barley of the Sonet variety under the action of the preparation "Naturost-M" exceeded the control by 7–10% (Table 5; Fig. 4). We should say that the grain productivity of the barley was lower in comparison with more favorable 2019 and 2022 crops under the wet and cool conditions experienced in 2020.

The grain productivity of wheat in the small-plot experiment under the action of the studied preparation exceeded the control by 5–11%. It is important to mention that the growth in grain productivity of both varieties of oat plants under the action of the preparation was similar. For example, grain productivity increased by 15–31% in plants of the Lev variety compared to the control and in the Yakov variety by 12–31%. In this case, the effectiveness of action on the grain productivity of oats in the bacterial *B. megaterium* B-4801 strain was generally more significant in the warm and moderately humid summer of 2022 and less in the hot and dry of 2021.

The assessment of yield structure demonstrated that the barley of the Sonet variety under the action of the experimental preparation "Naturost-M", based on bacterial *B. megaterium* B-4801 strain, had an increase in the number of productive shoots by 9–10% and grain weight by 3%, while the number of grains in the ear remained at the control level. In oat plants, regardless of variety, the most significant contribution to the change in grain productivity under the action of the tested preparation was made by the number of grains in the panicle (the difference with the control reached 14% in the Yakov variety and 11% in the Lev variety), while the grain weight, in general, was at the level of control (the difference with the control amounted to 1–6%). The preparation affected the grain productivity of the wheat of the Daria variety mainly through the change in grain weight by 5–11% and growth in the number of productive shoots by 9–10%.

4. Conclusions

As a result of the genome annotation of the studied *B. megaterium* strain B-4801, we identified the main components of antimicrobial compound biosynthesis pathways, including the cluster of genes (*FabD*, *FabF*, *FabG*, *FabI*, *FabZ*, etc.) responsible for the synthesis of enzymes for the formation of aliphatic unsaturated carboxylic acids containing 3–18 carbon atoms (butyric, lauric, caproic, capric, caprylic, palmitic, myristic, stearic, and oleic acids). We also revealed genetic loci encoding the synthesis of bacteriocins such as canosamine and polyketide ansamycin bacteriocins. Interestingly, the genome of the *B. megaterium* B-4801 strain studied by us contained clusters responsible for the biosynthesis of such secondary metabolites as siderophores and lantipeptides, as well as a whole range of genes responsible for various adaptation mechanisms of the strain to environmental conditions. According to the chromatographical analysis of the culture fluid of the *B. megaterium* B-4801 strain, its main components are butyric acid and the corresponding hydroxy derivatives with relative contents of about 27% and 14%, respectively. In addition, the strain could synthesize aspartic and glutamic acids and riboflavin. Therefore, the genome and metabolome characteristics of the *B. megaterium* B-4801 strain we obtained allow us to assume a wide range of antimicrobial ac-

tivity resistance to adverse factors and recommend it for plant growth stimulation. The experimental preparation "Naturost-M", based on the *B. megaterium* B-4801 strain, showed its effectiveness in crop production in Russia's non-Chernozem zone conditions. For example, the treatment of cereal crops with the preparation "Naturost-M" contributed to the increase in growth parameters: raw weight was increased to 67% compared to the control, and dry weight was increased up to 79% (depending on the year of study, phase of ontogenesis, and crop), which occurred against the background of growing content of photosynthetic pigments. Grain productivity under conditions of small-plot experiments in variants with the preparation increased barley by 7–46%, oats by 12–31%, and wheat by 5–11%.

Abbreviations

G/C, guanine/cytosine; RNA, ribonucleic acid; DNA, deoxyribonucleic acid.

Availability of Data and Materials

All data points generated or analyzed during this study are included in this article. Any additional information will be provided upon request to the corresponding author.

Author Contributions

AP, IR, and GL developed the experiment program, AP, IR and LI conducted the experiment, IR, LS, LI and EB conducted laboratory analyses, IR, AP and GL analyzed the data, AP, IR and LI wrote the original manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity. All authors contributed to editorial changes in the manuscript.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

The authors thank BIOTROF LLC for providing the biopreparation for the study. The authors thank the reviewers for their opinions and suggestions.

Funding

This research received no external funding.

Conflict of Interest

All authors declare no conflicts of interest. Despite receiving sponsorship from BIOTROF LLC, the judgments in data interpretation and writing were not influenced by this relationship.

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