agronomy

MDPI

Article

The Multiple Activities and the Plant Beneficial Potential of *Bacillus* spp. Derived from the Permafrost of Western Siberia

Olga V. Domanskaya [1], Nina A. Bome [2], Aleksandr V. Iashnikov [1], Anastasia V. Vasilchenko [1,3] and Alexey S. Vasilchenko [1,*]

1 Laboratory of Antimicrobial Resistance, Institute of Environmental and Agricultural Biology (X-BIO), Tyumen State University, Tyumen 625003, Russia; olga-nv@bk.ru (O.V.D.); yashnikov1998@inbox.ru (A.V.I.); vasilchenko.av.83@gmail.com (A.V.V.)
2 Biology Institute, Tyumen State University, Tyumen 625003, Russia; bomena@mail.ru
3 All-Russian Institute of Plant Protection, St. Petersburg-Pushkin 196608, Russia
* Correspondence: avasilchenko@gmail.com

Abstract: Agents of biological control are an important part of traditional agriculture, as well as organic farming. However, in the climatic conditions of countries that are located in cold and temperate regions, plant protection requires particular biocontrol agents that have adapted to environments with low and unstable temperatures. This work presents the biocontrol potential and plant-promoting activity of *Bacillus* spp. that was isolated from permafrost sediments in Western Siberia. It was found that all of the studied strains (n = 10) were able to produce indole-3-acetic acid (IAA) and chitinolytic enzymes at low positive temperatures (5 °C). The antifungal activity of cold-tolerant bacilli against *Microdochium* sp., *Fusarium* spp., and *Alternaria* sp was recorded. In greenhouse and field conditions, the selected strains (*B. simplex* 948P-1 (IAA-producing) and *B. megaterium* 312 (with antifungal activity)) were assessed in comparison to a commercially available fungicide (tebuconazole) and biofungicide (*B. subtilis* 26D). It was found that the bacilli in the seed germination assay exhibited low phytotoxicity and there was no significant advantage over the conventional fungicides in the yield stimulation assay. However, the twin consortia of *B. megaterium* 312 and *B. simplex* 948P-1 was able to increase winter wheat yields by 50% (compared to the untreated group), and by 70% (compared to the commercial biofungicide-treated group). Moreover, applying the twin consortia of *Bacillus* spp. significantly reduced the infection rate of *Fusarium* spp. in first-generation wheat grain.

Keywords: cold-adapted biopesticides; biocontrol; growth-promoting activity; plant protection; bacillus consortium; Siberia; permafrost

Citation: Domanskaya, O.V.; Bome, N.A.; Iashnikov, A.V.; Vasilchenko, A.V.; Vasilchenko, A.S. The Multiple Activities and the Plant Beneficial Potential of *Bacillus* spp. Derived from the Permafrost of Western Siberia. *Agronomy* **2021**, *11*, 2347. https://doi.org/10.3390/agronomy11112347

Academic Editors: Eligio Malusá, Xiangming Xu and Ivana Maksimović

Received: 7 November 2021
Accepted: 17 November 2021
Published: 19 November 2021

1. Introduction

The bioclimatic potential of different northern regions is not homogeneous and is considered less favorable for the cultivation of various crops. For example, in the European Union (EU), the regional cropland areas typically comprise 0 to 25 percent of the total land area [1,2].

It is possible to highlight some of the features of crop production in cold regions. First, the growth and productivity of many economically important plant species are sensitive to the temperature conditions. A periodic decrease in temperatures to the nonfreezing level (0–15 °C) causes chilling stress in plants [3]. The general symptoms that occur in response to chilling injuries in horticultural plants are surface lesions, discoloration due to loss of chlorophyll, plant death, and accelerated senescence [4].

Secondly, the development of sustainable crop production in the northern regions, as well as in the southern regions, is adversely affected by the natural and climatic conditions that contribute to the development of phytopathogens. As a result, this causes a significant decrease in yield. It is known that soil fungi can remain physiologically active during winter [5]. For example, phytopathogenic fungi *F. subglutinans* are widespread in the

northern regions of Europe under colder and more humid conditions [6]. In addition to fungi, the cold-tolerant pathogenic *Pseudomonas* spp. also damages the crops [7]. They serve as nuclei for ice formation and, therefore, cause frost injuries to plants at temperatures below 0 °C [7]. In addition, as a result of climate change, there is the tendency for plant pathogens to spread from southern to northern territories [8,9].

At present, chemical plant protection products are most commonly used against the diseases and pests that affect agricultural crops. The use of fungicides is an expensive and ineffective means of controlling fungal etiology diseases under cold climatic conditions. This is due to the fact that chemical reactions are ineffective in cold conditions and higher doses are required compared to more southern regions [10]. The degradation of synthetic pesticides in cold climates is very slow [11].

Considerable efforts are being made to solve the problem of cold stress in plants, as well as to combat the phytopathogens of the important crops. To date, an impressive amount of work has accumulated on the isolation and characterization of new biological control agents and plant growth-promoting (PGP) microorganisms [10,11]. Cold-tolerant and psychrophilic microorganisms that produce phytostimulators and antimicrobial secondary metabolites could be used for improving plant growth, fighting phytopathogens, and helping to increase yields, even in adverse temperature conditions. However, the PGP potential of cold-adapted microorganisms is less understood [12–15].

Developing the biological methods for controlling pathogenic fungi involves identifying and isolating the producers of biologically active substances in low temperature habitats, which are highly competitive in biocenosis. The bacteria of the genus *Bacillus* are spore-forming microorganisms that are capable of growing in a wide range of temperatures, UV radiation levels, and nutrient availability levels, which makes them ubiquitous creatures that can survive even in the conditions of the Martian atmospheric environment [16]. For plant beneficial mesophilic strains of bacilli, as well as pseudomonads, there is the potential for a number of commercial preparations. In relation to widespread psychrotrophic pathogens in crop production, the question of finding the psychrophilic and psychrotrophic strain antagonists of phytopathogens is imperative.

Here, we assessed the biocontrol and plant growth-promoting potential of *Bacillus* spp. that were derived from the permafrost in Western Siberia, as characterized in our previous work [17]. The antifungal activity, and the production of chitinolytic enzymes and auxin, was evaluated at low positive temperatures. The phytotoxicity of promising strains was measured in greenhouse conditions, while in-field experiments with *Triticum aestivum* L. were carried out. The permafrost-derived bacilli showed the ability to increase the yield of winter wheat and to reduce the seeds' fungal infection rate.

2. Materials and Methods

2.1. Strains and Cultivation Conditions

The investigated strains of *Bacillus* spp. were isolated from the core of wells drilled in the Tarko-Sale region of Western Siberia (Russian Federation). The permafrost was located in the Upper and Middle Pleistocene epochs of the IV marine terrace. The phylogenetic position and growth conditions of the strains were described in our previous work [17]. The strains were deposited in the All-Russian Collection of Industrial Microorganisms (National Research Center "Kurchatov Institute", Russian Federation).

2.2. Screening and Selection of Active Isolates

2.2.1. Antifungal Activity

Bacterial strains were grown in LB medium (g/L: tryptone—10, yeast extract—5, NaCl—2). The strains were cultured at mesophilic and low temperature conditions (5 and 22 °C, respectively) and stirred in 250 mL bottles until the stationary phase was reached.

Antifungal activity was determined by applying the method of diffusion in agar [18] to phytopathogenic fungi belonging to *Alternaria* sp., *Fusarium avenaceum* (Fr.) Sacc., *Fusarium graminearum* (Schwabe), and *Microdochium nivale* (Fr) Samuels and I.C. Hallet from the

collection of the Laboratory of Biotechnological and Microbiological Research, Tyumen State University (Russian Federation).

Briefly, agar plates containing 20 mL of potato glucose agar (Sigma-Aldrich, St. Louis, MO, USA) were seeded with fungal spores. Wells were cut out of the agar plates and an aliquot of 20 µL of the bacterial suspension of each test organism (containing 10^8 CFU mL) was added to the well. After incubation at 25 °C for 7 days, the diameter of the zone of inhibition was measured. The bacterial strains were tested in at least three replicates in two independent experiments. The results are presented as mean values ± standard deviation.

2.2.2. Determination of Chitinase Activity

The total chitinase activity was determined by measuring the amount of reducing sugars that formed as a result of the hydrolysis of colloidal chitin reacting with 2,3,5-triphenyltetrazolium chloride (TTZ) [19].

An inductor substrate (0.2% colloidal chitin (Sigma-Aldrich, St. Louis, MO, USA)) was used as a carbon source. To determine the chitinase activity, bacteria were grown in a minimal medium (g/L: Na_2HPO_4—6; KH_2PO_4—3; NH_4Cl—1; NaCl—0.5; yeast extract—0.05; colloidal chitin 1% (*w*/*v*)). To determine the optimal incubation temperature for chitinase production, cultivation was carried out at 5 and 22 °C.

The reaction mixture was prepared to contain 1 mL of culture supernatant and 0.3 mL of 0.2 M Na-phosphate buffer. The obtained supernatant was mixed with 0.2 mL of colloidal chitin and the mixture was incubated for 1 h at 37 °C. Thereafter, the tubes were centrifuged at 8000× *g* for 10 min. The resulting supernatants (750 µL) were mixed with 0.5 µL of TTZ, and the mixtures were heated in a water bath at a temperature of 100 °C for 5 min. Then, the mixtures were cooled and 10 mL of ethanol (96%) was added. The absorbance of the reaction mixture was measured at 485 nm using a Multiskan Go plate-reader (Thermo Scientific, Waltham, MA, USA). The unit of activity was converted in the enzyme, under the action of which 1 µM N-acetylglucosamine was formed within 1 h under the reaction conditions. The bacterial strains were tested in at least three replicates in two independent experiments. The results are presented as the mean values ± standard deviation.

2.2.3. Determination of Indole-3-Acetic acid Production

The strains were cultured in LB medium supplemented with L-tryptophan (Sigma-Aldrich, St. Louis, MO, USA) at a concentration of 1 g/L as an indole-3-acetic acid (*IAA*) precursor. The cultivation was carried out in a batch mode with stirring at 140 rpm at temperatures of 5 and 22 °C. The IAA content in the culture suspension was estimated using Salkowski reagent, according to [20,21]. All the studies were repeated on three independent dates to confirm the results. The bacterial strains were tested in at least three replicates in two independent experiments. The results are presented as the mean values ± standard deviation.

2.2.4. Design and Set Up of the Greenhouse Experiments

A paper roll towel bioassay was used to determine the influence of the studied *Bacillus* spp. strains on the growth and development of wheat seeds (*Triticum aestivum* L.) [22,23]. Bacterial cultures were obtained via cultivation for a week at 5 °C and 24 h at 22 °C. The cultures were diluted with LB medium to optical density corresponding to 10^8 CFU/mL. Before sowing, the seeds were surface sterilized, then inoculated in bacterial suspensions for 2 h, and dried in the air. The seeds were germinated in rolls of filter paper at a temperature of 22 °C. Control seeds inoculated with sterile water served as a negative control.

The commercially available conventional preparations served as positive controls. One of them was Raxil Ultra (Bayer, Leverkusen, North Rhine-Westphalia, Germany), which is a tebuconazole-containing fungicide; another was Phytosporin-M (Russian Federation), which contains the spores of *Bacillus subtilis* 26D.

The growth-stimulating effect of the bacterial strains was assessed by observing changes in the morphometric parameters of wheat seeds (*Triticum aestivum* L.) on the 14th day of the experiment.

Each variant of the experiment included four replicates of 50–200 seeds each.

2.2.5. Design and Set Up of the Field Experiments

Microorganisms were cultured in an LB nutrient medium with shaking (180 rpm) at 22 °C for 96 h. Then, the biomass of microorganisms was separated by centrifugation for 10 min at 8000× *g*, and the supernatant was diluted with distilled water to obtain a concentration of 10^8 CFU/mL.

The wheat seeds were soaked in a bacterial suspension for 2 h. The seeds were treated with Phytosporin-M and Raxil Ultra according to the manufacturer's recommendations. The control seeds were soaked in sterile water.

Field experiments were carried out at the biological station "Kuchak Lake" of Tyumen State University. The experimental site of the biological station is located in the Nizhnetavdinsky district of the Tyumen region on the border of agroclimatic zones: subtaiga and northern forest–steppe (57°12′20.3″ N, 66°01′56.6″ E).

Sowing was carried out in the third week of August. The field experiment was laid on plots with a counting area of 1 m^2, and the distance between rows was 20 cm. The seeding rate was 400 seeds per plot. The number of sowing lines was 4. The depth setting was 5 cm.

Winter wheat was harvested in the full ripeness phase. The effects of different treatments were evaluated postharvest. Characteristics such as the weight of 1000 grains, plant height, the number of productive stems, the length of the main spike, the number of grains in the spike, and the weight of the grain per spike were considered.

2.2.6. Assessment of Wheat Yield

The first generation of *Triticum aestivum* L. seeds was used in the assay (n = 90). The seeds were sterilized with 0.5% sodium hypochlorite for 5 min, washed three times with sterilized distilled water, and placed on potato-glucose agar (Sigma-Aldrich, St. Louis, MO, USA). The Petri dishes were incubated at 20 °C for 5 days. The appearance of the formed fungal colonies was recorded. The seed infection rate was calculated by the formula according to [24]:

Effect (%) = (seed infection rate of the control group–seed infection rate of the treatment group)/seed infection rate of the control group × 100.

Fungi were identified based on morphological characteristics, as described in [24].

2.2.7. Statistical Processing

The obtained results were statistically manipulated using Origin 2021 (OriginLab Corporation, Northampton, MA, USA) software.

The Shapiro–Wilk test was used to assess the normality of value distributions. In the presence of a normal distribution, the Student's *t*-test was used; if the normality was rejected, the nonparametric paired sample Wilcoxon signed-rank test was applied. Differences were considered significant at a *p*-value of ≤ 0.05. The data were presented as the mean and standard deviation (mean ± SD).

3. Results

3.1. Effect of Cultivation Temperature on Antifungal Activity of the Permafrost-Derived Bacillus spp.

Among the studied strains, only *B. megaterium* 312 and *B. cereus* 875 demonstrated antifungal activity (Table 1). When cultured at 5 °C, antifungal activity was shown in both *B. megaterium* 312 and *B. cereus* 875, while at the higher temperature, only *B. cereus* 875 inhibited fungal growth (Table 1).

Table 1. Antifungal activity of the strains of *Bacillus* spp. depending on the cultivation temperature.

Strains	t °C	Growth Inhibition Zones, mm			
		***Alternaria* sp.**	***F. avenaceum***	***F. graminearum***	***M. nivale***
B. megaterium 312	5	21.0 ± 2.12	17.0 ± 1.41	13.0 ± 1.06	23.0 ± 0.14
	22	0	0	0	0
B. cereus 875	5	0	0	0	0
	22	0	5.0 ± 1.14	8.0 ± 2.8	0

3.2. Chitinolytic Activity of Psychrotolerant Bacillus spp.

One of the factors of antagonism against phytopathogenic fungi is the synthesis of the chitinolytic enzymes that are involved in the lysis of fungal cells.

All of the studied strains were able to produce chitinolytic enzymes (Figure 1). However, some of them had a temperature optimum and minimum for chitinolytic activity to take place. It was found that the temperature optimum was 5 °C for six strains. This included *B. cereus* 875 (1.62 µg/mL h), *B. subtilis* B-10130 (1.27 µg/mL h), and *B. cereus* 1562 TS (0.97 µg/mL h). Four strains had a temperature optimum of 22 °C for chitinase production, including *B. simplex* 948 P1 (1.13 µg/mL h) and *B. subtilis* 948P (0.98 µg/mL h).

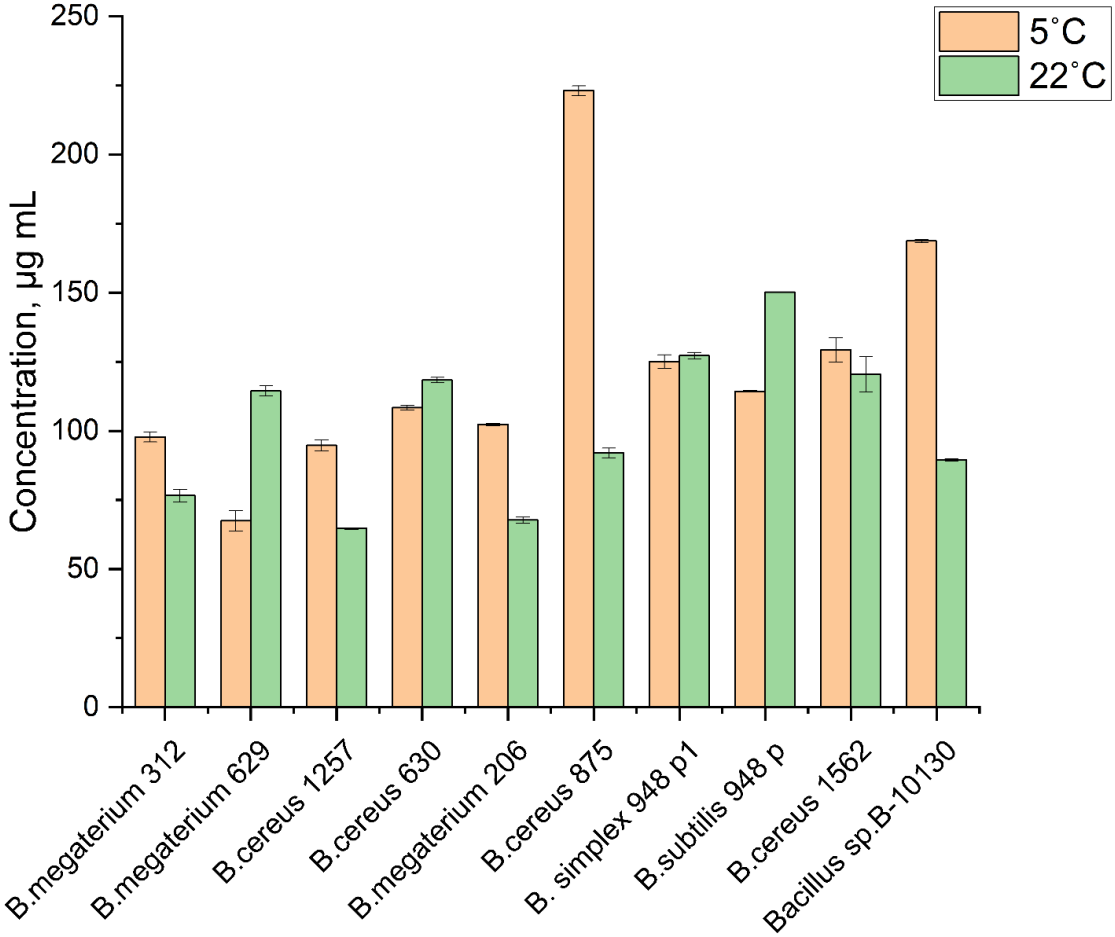

Figure 1. Influence of the cultivation temperature on the chitinolytic activity of the psychrotolerant *Bacillus* spp strains. The units of chitinolytic activity were converted in the enzyme, under the action of which 1 µM N-acetylglucosamine is formed within 1 h.

3.3. Auxin-Producing Activity in the Permafrost-Derived *Bacillus* spp.

The next stage of the work was selecting the strains that were capable of synthesizing phytohormone indole-3-acetic acid (IAA). All of the studied *Bacillus* spp. strains were generally capable of producing IAA over a wide range of culturing temperatures. However, the highest quantitative yield of phytohormone (27.5–95.2 μg/mL) was observed during cultivation at 22 °C (Table 2). A cultivation temperature of 5 °C was favored by strains *B. cereus* 875 (11.5 μg/mL), *B. cereus* 1257 (14.7 μg/mL), and *B. simplex* 948 P1 (16.0 μg/mL).

Table 2. IAA content in the culture liquid of the studied bacterial strains, μg/mL.

Strains	Cultivation Temperature	
	5 °C	**22 °C**
B. simplex 948 P1	16.0 ± 3.6	95.2 ± 10.8
B. cereus 1257	14.7 ± 2.4	4.0 ± 2.22
B. cereus 875	11.5 ± 0.8	5.2 ± 0.53
Bacillus sp.B-10130	8.8 ± 1.3	28.8 ± 5.8
B. cereus 1562	8.5 ± 0.9	28.0 ± 4.1
B. megaterium 206	7.8 ± 1.5	5.2 ± 0.7
B. megaterium 312	7.7 ± 0.8	17.0 ± 2.9
B. cereus 630	6.8 ± 0.6	58.0 ± 1.07
B. subtilius 948P	6.5 ± 0.9	4.5 ± 0.5
B. megaterium 629	5.0 ± 0.8	27.5 ± 6.2

3.4. Growth-Stimulating Activity of Psychrotolerant *Bacillus* spp.

To assess the manifestation of inhibiting or stimulating activity, two strains were selected. One strain, *B. simplex* 948P-1, previously showed high auxin-producing activity, while *B. megaterium* 312 showed antifungal activity at low positive temperatures. The activity of psychrotolerant *Bacillus* strains was assessed against commercial preparations of biopesticide (Phytosporin-M, containing spores of *B. subtilis* 26D) and chemical fungicide (Raxil Ultra, containing tebuconazole).

The results of the greenhouse experiments showed that different treatment options for wheat seeds influenced the growth parameters of the plants. The results of the laboratory experiments showed that, in all but one (Raxil Ultra) of the experimental groups, treatment significantly increased the length of the root and shoot compared to the control (Figure 2). Thus, the shoot length was most favorably influenced by treatment with *B. simplex* 948P1 (Figure 2a), while treatment with Raxil Ultra reduced the recorded values by more than 30%. The accumulation of root biomass was most stimulated by treatment with a combined biocontrol agent (BCA) preparation (*B. simplex* 948 P1 combined with *B. megaterium* 312) (Figure 2b), while the shoot biomass was most stimulated with Phytosporin-M treatment, compared to the control group (Figure 2b).

In general, it should be noted that seed treatment with bacteria from the permafrost and the biological commercial product Phytosporin-M led to an increase in the main growth parameters of the plants.

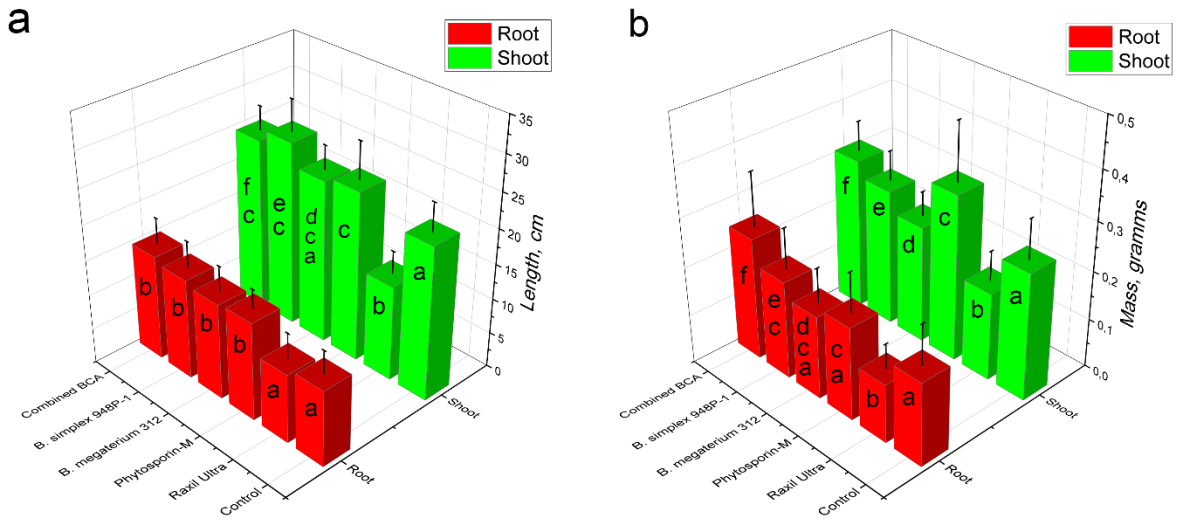

Figure 2. Influence of strains of the genus *Bacillus* spp. on the morphometric parameters of wheat seedlings (Triticum aestivum L.). The length (**a**) and biomass (**b**) of wheat seedlings were assessed by measurements on the 14th day of the treatment. Treatments not connected by the same letter in each bar are significantly different from each other ($p < 0.05$; means $\pm$SD).

3.5. Assessment of Wheat Yield

The productivity of winter wheat is largely determined by the nature of the growth and development of plants during the growing season. A significant contribution is made to the formation of the yield by the stem as a photosynthetically active organ, as well as an organ for the temporary supply of substances.

Our study showed that pre-sowing seed treatments with the chemical fungicide Raxil Ultra, the biological preparation Phytosporin-M, and the permafrost-derived bacterial strains had a different effect on the yield of winter wheat.

The analysis of the grain yield showed that pickling the seed with the chemical fungicide "Raxil Ultra" did not significantly affect the indicator values (Table 3). It is interesting that in the "Phytosporin-M", *B. simplex* 948 P1, and *B. megaterium* 312 groups, the grain yield was slightly lower than it was in the control group. In turn, an increase in yield was recorded when the seeds were treated with a suspension of a consortium of the strains *B. simplex* 948P-1 and *B. megaterium* 312 (combined BCA), which made it possible to obtain a yield increase of 50.2%, compared to the control group (Table 3).

Table 3. The influence of the treatment of winter wheat seeds with various preparations and bacterial strains on the characteristics of productivity.

Experimental Groups	The Number of Grains per Ear, pcs.	Weight of 1000 Grains, g	Yield, g/m^2
Control	45.8 $\pm$ 11.70	38.31	395.23
Raxil-Ultra	42.73 $\pm$ 9.63	35.9	400.40
Phytosporin-M	42.67 $\pm$ 9.66	35.72	331.20
B. megaterium 312	38.26 $\pm$ 9.74	38.93	357.86
B. simplex 948P-1	41.27 $\pm$ 2.46 *	34.51	285.12
combined BCA	46.13 $\pm$ 8.57	39.73	593.74

Asterisks show statistically significant differences with a control at $p < 0.05$ (n = 400).

3.6. Phyto-Examination of the First Generation Seeds

The phyto-examination of the first generation winter wheat seeds revealed the presence of mycotoxin-forming fungi belonging to the genera *Alternaria* spp., *Fusarium* spp., and *Helminthosporium* spp.

The largest number of first generation seeds were affected by the causative agent of the moles of cereals (*Alternaria* spp). Thus, in the control group, more than half of the seeds were damaged (62.3%). Less damage was observed in the plants that were treated with Raxil Ultra (37.6%), B. simplex 948P-1 (34.2%), and B. megaterium 312 (30.8%). Seeds from the combined BCA (26.5%) and Phytosporin-M (27.5%) groups were the least affected by *Alternaria* spp. (Figure 3).

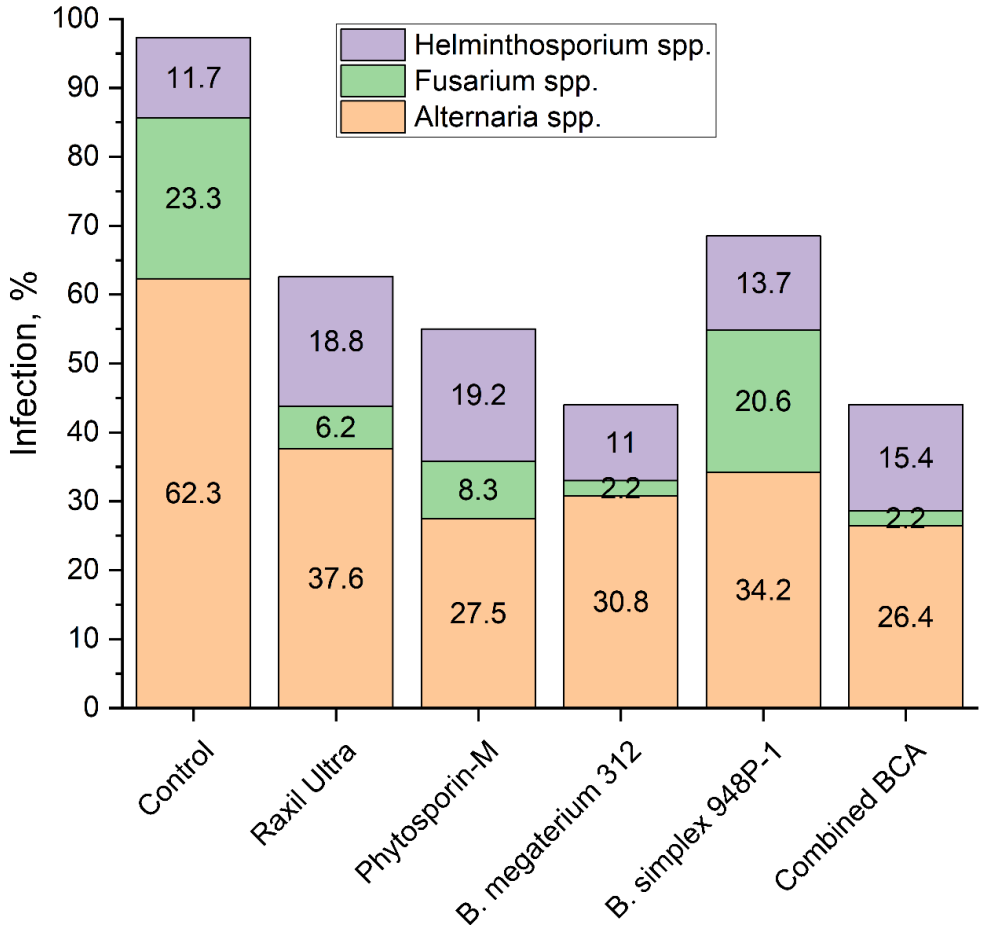

Figure 3. Damage to seeds of the first generation of winter wheat *Triticum aestivum* L.

The infection rate of winter wheat grain with *Helminthosporium* spp. ranged from 11.7 to 19.2%. The highest percentage of infected seeds appeared in the groups that were treated with Raxil Ultra (19.8%) and Phytosporin-M (19.2%). The most resistant plants were those that were treated with *B. megaterium* 312 (11%) and those in the control group (11.2%) (Figure 3).

An important indicator that characterizes the phytosanitary state of grain crops is seed infection with *Fusarium* spp. According to the phyto-examination results, the most infected wheat grain was in the group that was treated with *B. simplex* 948 P1 (20.6%), while the least infected wheat grain was in the variants that were treated with *B. megaterium* 312 (2.2%) and combined BCA (2.2%) (Figure 3).

For phytosanitary control, we used seeds (first generation) that were obtained by growing wheat seeds of *Triticum aestivum* L, treated (zero generation) with the studied *Bacillus* spp. strains. The evaluation of seed infestation was carried out via microscopic examination (n = 100).

4. Discussion

Climate change is a driver for all aspects of human activity. The increasing temperature on Earth has led to melting glaciers and permafrost zones. This process has both negative and positive consequences for humanity in terms of biosecurity. On the one hand, the thawing of permafrost zones opens up novel pathogens [25,26]; on the other hand, permafrost is a source of new microorganisms that may be useful for humans [27]. One of these bacteria, identified as *Bacillus cereus* strain BF, has been found in frozen Miocene sediments and has been proposed for probiotic formulation [28]. In general, bacteria that are isolated from permafrost are psychrophiles and psychrotolerant microorganisms, which allows them to be used in temperate regions. Moreover, a significant part of the Earth is subjected to short-term cold periods, which affect the efficiency of various microbial processes, such as nitrogen fixation, plant protection activity, and others [29]. From the perspective of biocontrol agents, ancient microorganisms may have advantages over other bacteria. However, to our knowledge, there is no report that focuses on the plant growth-promoting activity or plant protection ability of permafrost-derived bacteria.

In this work, the plant-promoting and plant protection activities of permafrost-derived *Bacillus* spp. strains were studied. The *Bacillus* strains were chosen due to their ability to produce catalase, dehydrogenase, amylolytic, proteolytic, and lipolytic enzymes under low positive temperatures [17]. The bacteria of the genus *Bacillus* are perhaps the largest group of microorganisms that produce antimicrobial metabolites and, therefore, are widely used as biopesticides [30].

The assessment of antifungal activity revealed that the strains *B. megaterium* 312 and *B. cereus* 875 were capable of inhibiting the growth of phytopathogenic fungi *Alternaria* sp., *Fusarium avenaceum*, *Fusarium graminearum*, and *Microdochium nivale*. The spectrum of antifungal antibiotics that are produced by *Bacillus* spp. is wide, including volatiles, siderophore, bacteriocins, lipopeptides, polyketides, and others [31]. Among them, lytic enzymes (chitinases) are of particular importance, since chitin is an abundant biogenic molecule that is present in both fungi and pest insects. This determines the potential for chitinase-producing bacteria to be multifunctional biocontrol agents exhibiting both fungicidal and insecticidal properties [32]. The permafrost-derived *Bacillus* spp. chitinase activity has some unique features. It was found that the chitinase activity of *Bacillus* spp. was maximal at a low positive temperature (67–223 μg/mL). This is an unusual result, since the optimum state for chitinase activity is at higher temperatures [32–35].

It is important to select potential bioagents not only based on the presence of antifungal activity, but also on their ability to have a positive effect on the growth and development of plants. Among the substances that stimulate plant growth, auxin is the most widespread. Auxin-producing microorganisms can impact plants in two ways. On the one hand, microbial auxin can interfere with plant development by disturbing the auxin balance in plants; on the other hand, it can stimulate plant root development [36]. It is known that when receiving an additional amount of auxin from PGP bacteria (plant growth-promoting), a plant causes transcriptional changes in the genes involved in plant defense [37]. Moreover, a lot of focus has been on microbial-produced IAA as a signaling molecule modulating the behavior of neighboring bacteria [38].

It is important to note that plants usually produce less auxin [39] at low temperatures, and the cold decreases the auxin accumulation in plant tissues [40]. In this regard, the ability of cold-tolerant bacteria to produce auxin at low temperatures is highly beneficial for plant growth-promoting (PGP) activity.

The strains *B. simplex* 948P, *B. megaterium* 312, and *B. cereus* 630 that were obtained from the permafrost were capable of producing auxin at low temperatures (5 °C) at a level of 17 to 95 μg/mL, depending on the strain. This is even higher than the known cold-tolerant PGP strain *Pseudomonas* sp. strain PGERs17 from the Himalaya region of India, which was found to produce only 1.4 μg/mL at 4 °C [22], or *Pantoea dispersa* 1A and *Serratia marcescens* SRM from the northwestern Indian Himalayas, which were found to produce 3.0 μg/mL and 8.1 μg/mL, respectively [41].

Comparing the results, we can note that the level of auxin synthesis was several times higher in the bacteria of the genus *Bacillus* that was isolated from the permafrost, not only compared to cold-tolerant bacteria, but to mesophilic as well [42–44].

When selecting the potential strains for protecting agricultural plants from phytopathogens, both the manifestation of antifungal activity and the positive effect on the growth and development of plants were taken into account. For the greenhouse experiments, five bacterial strains were selected according to a set of favorable indicators (antifungal activity and high auxin synthesis at 5 °C). The result of the greenhouse experiment was suggesting three strains as potential biocontrol agents (*B. cereus* 875, *B.megaterium* 312, and *B. simplex* 948P) that had a pronounced growth-stimulating effect on cereal plants (*Triticum aestivum* L). The use of biopesticides is criticized in terms of the safety of their use [45]. Although biopesticides can be considered a "low-risk active substance" (Article 22 of EC 1107/2009) [46,47], tests for cytotoxicity or genotoxicity are performed as a preliminary risk assessment before proceeding with licensing [47]. Bioherbicides based on *Trichoderma koningiopsis* demonstrated high phytotoxic effects on weeds, but low genotoxocity [48]. Insecticidal biopesticides based on *Bacillus thuringiensis* did not produce mutagenic effects in bone marrow or sperm cells [49]. In this regard, the permafrost-derived *Bacillus* spp. studied in this work showed the absence of toxic effects on wheat seeds in the seed germination assay. In addition, these bacilli had no genotoxic effect on the cells of the apical meristem of oat seedlings (Supplementary Materials). Interesting results were uncovered regarding the presowing seed treatment using the commercial preparations of the fungicide Raxil Ultra and the biological commercial preparation Phytosporin-M in comparison to the bacterial strains from the permafrost. Thus, the chemical fungicide, as expected, did not affect the yield of winter wheat, while the studied bacterial preparations (including the conventional preparation) had weak inhibitory effects on their own.

In turn, seed dressing with a combined preparation (including the growth-promoting strain *B. simplex* 948P and the antifungal active strain *B.megaterium* 312) led to an increase in plant productivity by 50% compared to the control. Moreover, the treatments with this consortium led to reducing the infection rate of first generation wheat seeds. Previous research has shown that microbial consortia work better as biocontrol agents than individual strains [50]. The inoculation of plants with bacterial consortia can reduce the negative influence of biotic or abiotic stress conditions on crops [50]. This is illustrated well by the work of Saikia et al., which used a consortium of three bacterial strains that were involved in the plant's ethylene level. The achieved effect was an increase in the seed germination percentage, root length, shoot length, and the dry weight of the treated plants [51]. Another example demonstrated the resistance-promoting activity of a twin bacterial consortium of *Pseudomonas putida* and *Bacillus subtilis* against the soil-borne fungus *Macrophomina phaseolina* [52].

Among the pathogenic microflora of grain crops, the infection of seeds by specific fungi is an issue that is of particular importance. Moreover, the mycotoxins that are present in grain make it unsuitable for consumption. Thus, it must be noted that the seed quality directly determines the quality of agricultural products.

5. Conclusions

Previous studies have shown that permafrost can not only be a potential threat to human biosafety, but that it can also be a source of new beneficial microorganisms. The bacteria of the genus Bacillus have, once again, shown that they are not only able to exist in a wide range of environmental conditions, but that they can also produce a number of important biologically active metabolites. Permafrost-derived bacilli from Western Siberia is quite capable of competing with other known cold-adapted *Bacillus* spp. strains isolated from cold regions of the Earth.

The obtained results allowed an effective consortium of permafrost-derived *Bacillus* spp. to be formed, which are able to increase the yield of winter wheat and prevent the spreading of phytopathogenic fungi in wheat grains at low temperatures.

Thus, bacterial strains of *Bacillus* spp. that have been isolated from permafrost perform an activity that is similar to phytopathogens and are capable of biosynthesizing chitinolytic enzymes and auxin (in a wide temperature range). They can be considered as potential sources for the creation of biological products to be used for plant growth in the extreme conditions of northern territories.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11112347/s1; Table S1: Mitotic index of oat seedling root meristem cells under the treatment *Bacillus* spp.

Author Contributions: Performing experiments O.V.D., A.V.I., A.V.V.; methodology O.V.D., N.A.B.,A.S.V.; formal analysis O.V.D., N.A.B., A.V.V., A.S.V.; supervision A.S.V.; writing—original draft preparation, O.V.D., A.S.V.; writing—review and editing A.S.V. All authors have read and agreed to the published version of the manuscript.

Funding: The work was supported by the Russian Science Foundation (grant no. 19-76-30005).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ewert, F.; Rounsevell, M.; Reginster, I.; Metzger, M.; Leemans, R. Future scenarios of European agricultural land use: I. Estimating changes in crop productivity. *Agric. Ecosyst. Environ.* **2005**, *107*, 101–116. [CrossRef]
2. Peltonen-Sainio, P.; Hannukkala, A.; Huusela-Veistola, E.; Voutila, L.; Niemi, J.; Valaja, J.; Jauhiainen, L.; Hakala, K. Potential and realities of enhancing rapeseed- and grain legume-based protein production in a northern climate. *J. Agric. Sci.* **2013**, *151*, 303–321. [CrossRef]
3. Ruelland, E.; Collin, S. Chilling stress. In *Plant Stress Physiology*; Shabala, S., Ed.; CABI Publishing: Wallingford, UK, 2011; pp. 94–118.
4. Subramanian, P.; Kim, K.; Krishnamoorthy, R.; Mageswari, A.; Selvakumar, G.; Sa, T. Cold Stress Tolerance in Psychrotolerant Soil Bacteria and Their Conferred Chilling Resistance in Tomato (Solanum lycopersicum Mill.) under Low Temperatures. *PLoS ONE* **2016**, *11*, e0161592. [CrossRef]
5. Tibbett, M.; Sanders, F.; Cairney, J. Low-temperature-induced changes in trehalose, mannitol and arabitol associated with enhanced tolerance to freezing in ectomycorrhizal basidiomycetes (*Hebeloma* spp.). *Mycorrhiza* **2002**, *12*, 249–255. [CrossRef] [PubMed]
6. Goertz, A.; Zuehlke, S.; Spiteller, M.; Steiner, U.; Dehne, H.W.; Waalwijk, C.; de Vries, I.; Oerke, E.C. Fusarium species and mycotoxin profiles on commercial maize hybrids in Germany. *Eur. J. Plant Pathol.* **2010**, *128*, 101–111. [CrossRef]
7. Küdela, V. Potential impact of climate change on geographic distribution of plant pathogenic bacteria in Central Europe. *Plant Prot. Sci.* **2010**, *45*, S27–S32. [CrossRef]
8. Costanzo, J.P.; Lee, R.E. Supercooling and ice nucleation in vertebrate ectotherms. In *Biological Ice Nucleation and Its Applications*; American Phytopathological Society: St. Paul, MN, USA, 1995.
9. Barford, E. Crop pests advancing with global warming. *Nature* **2013**. [CrossRef]
10. Prank, M.; Kenaley, S.C.; Bergstrom, G.; Acevedo, M.; Mahowald, N.M. Climate change impacts the spread potential of wheat stem rust, a significant crop disease. *Environ. Res. Lett.* **2019**, *14*, 124053. [CrossRef]
11. Basu, A.; Prasad, P.; Das, S.; Kalam, S.; Sayyed, R.; Reddy, M.; El Enshasy, H. Plant Growth Promoting Rhizobacteria (PGPR) as Green Bioinoculants: Recent Developments, Constraints, and Prospects. *Sustainability* **2021**, *13*, 1140. [CrossRef]
12. Beneduzi, A.; Ambrosini, A.; Passaglia, L.M. Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. *Genet. Mol. Biol.* **2012**, *35*, 1044–1051. [CrossRef]
13. Qin, Y.; Fu, Y.; Kang, W.; Li, H.; Gao, H.; Vitalievitch, K.S.; Liu, H. Isolation and identification of a cold-adapted bacterium and its characterization for biocontrol and plant growth-promoting activity. *Ecol. Eng.* **2017**, *105*, 362–369. [CrossRef]
14. Pandey, A.; Yarzábal, L.A. Bioprospecting cold-adapted plant growth promoting microorganisms from mountain environments. *Appl. Microbiol. Biotechnol.* **2019**, *103*, 643–657. [CrossRef]
15. Wu, H.; Gu, Q.; Xie, Y.; Lou, Z.; Xue, P.; Fang, L.; Yu, C.; Jia, D.; Huang, G.; Zhu, B.; et al. Cold-adaptedBacilliisolated from the Qinghai–Tibetan Plateau are able to promote plant growth in extreme environments. *Environ. Microbiol.* **2019**, *21*, 3505–3526. [CrossRef] [PubMed]
16. Cortesão, M.; Fuchs, F.M.; Commichau, F.; Eichenberger, P.; Schuerger, A.C.; Nicholson, W.L.; Setlow, P.; Moeller, R. Bacillus subtilis Spore Resistance to Simulated Mars Surface Conditions. *Front. Microbiol.* **2019**, *10*, 333. [CrossRef] [PubMed]
17. Domanskaya, O.V.; Melnikov, V.P.; Ogurtsova, L.V.; Soromotin, A.V.; Domanskii, V.O.; Polyakova, N.V. Some features of enzyme activity in different strains of the Bacillus genus isolated from permafrost. *Earth's Cryosphere* **2017**, *XXI*, 53–59. [CrossRef]

18. Valgas, C.; De Souza, S.M.; Smânia, E.F.A.; Smânia, A., Jr. Screening methods to determine antibacterial activity of natural products. *Braz. J. Microbiol.* **2007**, *38*, 369–380. [CrossRef]
19. Rodriguez-Kabana, R.; Godoy, G.; Morgan-Jones, G.; Shelby, R.A. The determination of soil chitinase activity: Conditions for assay and ecological studies. *Plant Soil* **1983**, *75*, 95–106. [CrossRef]
20. Gordon, S.A.; Weber, R.P. Colorimetric Estimation of Indoleacetic Acid. *Plant Physiol.* **1951**, *26*, 192–195. [CrossRef] [PubMed]
21. Bric, J.M.; Bostock, R.M.; Silverstone, S.E. Rapid In Situ Assay for Indoleacetic Acid Production by Bacteria Immobilized on a Nitrocellulose Membrane. *Appl. Environ. Microbiol.* **1991**, *57*, 535–538. [CrossRef] [PubMed]
22. Mishra, P.K.; Mishra, S.; Selvakumar, G.; Bisht, S.C.; Bisht, J.K.; Kundu, S.; Gupta, H.S. Characterisation of a psychrotolerant plant growth promoting Pseudomonas sp. strain PGERs17 (MTCC 9000) isolated from North Western Indian Himalayas. *Ann. Microbiol.* **2008**, *58*, 561–568. [CrossRef]
23. Zhang, X.; Wang, R.; Ning, H.; Li, W.; Bai, Y.; Li, Y. Evaluation and management of fungal-infected carrot seeds. *Sci. Rep.* **2020**, *10*, 10808. [CrossRef]
24. Alexopoulos, C.J.; Mims, C.W.; Blackwell, M. *Introductory Mycology*, 4th ed.; John Willey and Sons Inc.: New York, NY, USA, 1996.
25. Houwenhuyse, S.; Macke, E.; Reyserhove, L.; Bulteel, L.; Decaestecker, E. Back to the future in a petri dish: Origin and impact of resurrected microbes in natural populations. *Evol. Appl.* **2018**, *11*, 29–41. [CrossRef]
26. Yarzábal, L.A.; Salazar, L.M.B.; Batista-García, R.A. Climate change, melting cryosphere and frozen pathogens: Should we worry . . . ? *Environ. Sustain.* **2021**, *4*, 489–501. [CrossRef]
27. Hough, M.; McClure, A.; Bolduc, B.; Dorrepaal, E.; Saleska, S.; Klepac-Ceraj, V.; Rich, V. Biotic and Environmental Drivers of Plant Microbiomes Across a Permafrost Thaw Gradient. *Front. Microbiol.* **2020**, *11*, 796. [CrossRef] [PubMed]
28. Brouchkov, A.; Melnikov, V.; Kalenova, L.; Fursova, O.; Pogorelko, G.; Potapov, V.; Fursova, N.; Ignatov, S.; Brenner, E.; Bezrukov, V.; et al. Permafrost Bacteria in Biotechnology: Biomedical Applications. In *Psychrophiles: From Biodiversity to Biotechnology*; Springer: Singapore, 2017; pp. 541–554.
29. Mishra, P.K.; Joshi, P.; Bisht, S.C.; Bisht, J.K.; Selvakumar, G. Cold-Tolerant Agriculturally Important Microorganisms. In *Plant Growth and Health Promoting Bacteria*; Springer: Singapore, 2010; pp. 273–296.
30. Jallouli, W.; Driss, F.; Fillaudeau, L.; Rouis, S. Review on biopesticide production by Bacillus thuringiensis subsp. kurstaki since 1990: Focus on bioprocess parameters. *Process. Biochem.* **2020**, *98*, 224–232. [CrossRef]
31. Caulier, S.; Nannan, C.; Gillis, A.; Licciardi, F.; Bragard, C.; Mahillon, J. Overview of the Antimicrobial Compounds Produced by Members of the Bacillus subtilis Group. *Front. Microbiol.* **2019**, *10*, 302. [CrossRef] [PubMed]
32. Bhattacharya, S.; DAS, A.; Samadder, S.; Rajan, S.S. Biosynthesis and characterization of a thermostable, alkali-tolerant chitinase from Bacillus pumilus JUBCH08 displaying antagonism against phytopathogenic Fusarium oxysporum. *3 Biotech* **2016**, *6*, 87. [CrossRef]
33. Martínez-Zavala, S.A.; Barboza-Perez, U.E.; Hernández-Guzmán, G.; Bideshi, D.K.; Barboza-Corona, J.E. Chitinases of Bacillus thuringiensis: Phylogeny, Modular Structure, and Applied Potentials. *Front. Microbiol.* **2020**, *10*, 3032. [CrossRef]
34. Senol, M.; Nadaroglu, H.; Dikbas, N.; Kotan, R. Purification of Chitinase enzymes from Bacillus subtilis bacteria TV-125, investigation of kinetic properties and antifungal activity against Fusarium culmorum. *Ann. Clin. Microbiol. Antimicrob.* **2014**, *13*, 35. [CrossRef]
35. Honda, S.; Kunii, T.; Nohara, K.; Wakita, S.; Sugahara, Y.; Kawakita, M.; Oyama, F.; Sakaguchi, M. Characterization of a Bacillus thuringiensis chitinase that binds to cellulose and chitin. *AMB Express* **2017**, *7*, 51. [CrossRef]
36. Spaepen, S.; Vanderleyden, J. Auxin and Plant-Microbe Interactions. *Cold Spring Harb. Perspect. Biol.* **2011**, *3*, a001438. [CrossRef] [PubMed]
37. Backer, R.; Rokem, J.S.; Ilangumaran, G.; Lamont, J.; Praslickova, D.; Ricci, E.; Subramanian, S.; Smith, D.L. Plant Growth-Promoting Rhizobacteria: Context, Mechanisms of Action, and Roadmap to Commercialization of Biostimulants for Sustainable Agriculture. *Front. Plant Sci.* **2018**, *9*, 1473. [CrossRef] [PubMed]
38. Keswani, C.; Singh, S.P.; Cueto, L.; García-Estrada, C.; Mezaache-Aichour, S.; Glare, T.R.; Borriss, R.; Singh, S.P.; Blázquez, M.A.; Sansinenea, E. Auxins of microbial origin and their use in agriculture. *Appl. Microbiol. Biotechnol.* **2020**, *104*, 8549–8565. [CrossRef]
39. Zhu, J.; Zhang, K.-X.; Wang, W.-S.; Gong, W.; Liu, W.-C.; Chen, H.-G.; Xu, H.-H.; Lu, Y.-T. Low Temperature Inhibits Root Growth by Reducing Auxin Accumulation via ARR1/12. *Plant Cell Physiol.* **2014**, *56*, 727–736. [CrossRef] [PubMed]
40. Gray, W.; Östin, A.; Sandberg, G.; Romano, C.P.; Estelle, M. High temperature promotes auxin-mediated hypocotyl elongation in Arabidopsis. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 7197–7202. [CrossRef]
41. Selvakumar, G.; Mohan, M.; Kundu, S.; Gupta, A.; Joshi, P.; Nazim, S.; Gupta, H. Cold tolerance and plant growth promotion potential of Serratia marcescens strain SRM (MTCC 8708) isolated from flowers of summer squash (Cucurbita pepo). *Lett. Appl. Microbiol.* **2007**, *46*, 171–175. [CrossRef]
42. Luziatelli, F.; Melini, F.; Bonini, P.; Melini, V.; Cirino, V.; Ruzzi, M. Production of Indole Auxins by *Enterobacter* sp. Strain P-36 under Submerged Conditions. *Fermentation* **2021**, *7*, 138. [CrossRef]
43. Chagas Junior, A.F.; De Oliveira, A.G.; De Oliveira, L.A.; Dos Santos, G.; Chagas, L.F.B.; da Silva, A.L.; Costa, J.D.L. Production of indole-3-acetic acid by Bacillus isolated from different soils. *Bulg. J. Agric. Sci.* **2015**, *21*, 282–287.
44. Swain, M.; Naskar, S.; Ramesh, R. Indole-3acetic acid production and effect on sprouting of yam (Dioscorea rotundata L.) minisetts by Bacillus subtilis isolated from culturable cowdung microflora. *Pol. J. Microbiol.* **2007**, *56*, 103–110. [PubMed]

45. Deising, H.B.; Gase, I.; Kubo, Y. The unpredictable risk imposed by microbial secondary metabolites: How safe is biological control of plant diseases? *J. Plant Dis. Prot.* **2017**, *124*, 413–419. [CrossRef]
46. Koch, E.; Becker, J.O.; Berg, G.; Hauschild, R.; Jehle, J.; Kohl, J.; Smalla, K. Biocontrol of plant diseases is not an unsafe technology! *J. Plant Dis. Prot.* **2018**, *125*, 121–125. [CrossRef]
47. Kiewnick, S. Practicalities of developing and registering microbial biologicalcontrol agents. *CAB Rev.* **2007**, *2*, 1–11. [CrossRef]
48. Camargo, A.F.; Venturin, B.; Bordin, E.R.; Scapini, T.; Stefanski, F.; Klanovicz, N.; Dalastra, C.; Kubeneck, S.; Preczeski, K.P.; Rossetto, V.; et al. A Low-Genotoxicity Bioherbicide Obtained from Trichoderma koningiopsis Fermentation in a Stirred-Tank Bioreactor. *Ind. Biotechnol.* **2020**, *16*, 176–181. [CrossRef]
49. Curbelo, A.; Mancebo, A.; Molier, T.; Arteaga, M.E.; González, C.; Grandía, R.; Bada, A.M.; Rivero, Y.; García, A.; Legró, M.; et al. Assessment of the in vivo genotoxicity of a new formulation of Bacillus thuringiensis var israelensis SH-14. *Toxicol. Environ. Chem.* **2011**, *93*, 691–699. [CrossRef]
50. Santoyo, G.; Guzmán-Guzmán, P.; Parra-Cota, F.I.; Santos-Villalobos, S.D.L.; Orozco-Mosqueda, M.D.C.; Glick, B.R. Plant Growth Stimulation by Microbial Consortia. *Agronomy* **2021**, *11*, 219. [CrossRef]
51. Saikia, J.; Sarma, R.K.; Dhandia, R.; Yadav, A.; Bharali, R.; Gupta, V.K.; Saikia, R. Alleviation of drought stress in pulse crops with ACC deaminase producing rhizobacteria isolated from acidic soil of Northeast India. *Sci. Rep.* **2018**, *8*, 3560. [CrossRef]
52. Sharma, C.K.; Vishnoi, V.K.; Dubey, R.; Maheshwari, D. A twin rhizospheric bacterial consortium induces systemic resistance to a phytopathogen *Macrophomina phaseolina* in mung bean. *Rhizosphere* **2018**, *5*, 71–75. [CrossRef]