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The Biocontrol of Plant Pathogenic Fungi by Selected Lactic Acid Bacteria: From Laboratory to Field Study

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Abstract: Plant diseases caused by pathogenic fungi generate large losses in crops and pose a threat to human and animal health. Since the European Green Deal put a strong emphasis on the need to reduce the use of chemical plant protection, interest in biological control has been growing. The present study aimed to investigate the efficacy of lactic acid bacteria (LAB) isolated from silages in the control of pathogenic fungi through in vitro, mini-plot, and field experiments. The tested LAB showed antifungal activity in vitro towards strains from the *Fusarium*, *Alternaria*, *Rhizoctonia*, *Colletotrichum*, and *Sclerotinia* genera; however, only five strains reached an activity ≥ 400 AU/mL towards all pathogenic fungi. The selected strains demonstrated high efficacy in reducing disease symptoms in plants in the mini-plot and field experiments. In the mini-plot experiment, stem smut of rye and wheat common bunt were reduced in the range 34.5–94.7% and 24.8–99.6%, respectively. In the field experiments, the efficacy of LAB in the control of rye and wheat disease differed and reached over 90% in some trials. The effectiveness of LAB in the control of seedling blight did not exceed 70%. A significant increase in yield (from 42.86 to 195.65%) was observed mainly in wheat cultivation. The increase in rye yield was observed only in chosen trials. No phytotoxicity was observed. The results indicate the potential possibilities of using LAB as a biocontrol agent.

Keywords: biological agents; biological control; integrated pest management; lactic acid bacteria; sustainable agriculture

Citation: Gwiazdowski, R.; Kubiak, K.; Juś, K.; Marchwińska, K.; Gwiazdowska, D. The Biocontrol of Plant Pathogenic Fungi by Selected Lactic Acid Bacteria: From Laboratory to Field Study. *Agriculture* **2024**, *14*, 61. https://doi.org/10.3390/agriculture14010061

Academic Editor: Yuan Li

Received: 3 November 2023
Revised: 20 December 2023
Accepted: 27 December 2023
Published: 28 December 2023

1. Introduction

In recent decades, there has been a growing interest in the use of biological methods in plant protection in the European Union (EU). One of the reasons is the need for agricultural producers to adapt to EU strategies aimed at reducing the use of pesticides. Directive 2009/128/EC of the European Parliament and the Council of 21 October 2009 obliged European Union countries to introduce the principles of integrated pest management as a part of sustainable agricultural production [1]. Moreover, the European Green Deal, announced in 2019 by the European Commission, puts a strong emphasis on the need to significantly reduce the use of chemical plant protection products [2]. Therefore, more emphasis has been placed on the development of non-chemical methods of plant protection such as biological methods, in which, among others, microorganisms can be used.

Biological control of plant diseases is understood as an environmentally friendly practice that means to suppress populations of plant pathogens with other organisms [3]. The concept of biocontrol is particularly important in the development of sustainable agriculture with lower ecological costs. Moreover, this concept is gaining interest due to increased awareness that the use of pesticides is associated with risks to human health and the environment. The health effects resulting from exposure to pesticides depend on the

type of pesticide, the duration and route of exposure, as well as the state of health. An important problem is the possibility of bioaccumulation of these substances in adipose tissue, although they can also be metabolized and excreted from the body. The negative health effects associated with chemical pesticides include dermatological, gastrointestinal, neurological, carcinogenic, respiratory, reproductive, and hormonal effects [4–6]. In the environment, the use of pesticides may reduce the biodiversity of organisms [7]. In contrast to chemical compounds, biopesticides are considered to be highly effective and eco-friendly, which is why research on them is being conducted very intensively. Biopesticides are agents based on living microorganisms or naturally occurring compounds that are used to control agricultural pests and pathogens. According to the United States Environmental Protection Agency, biopesticides are defined as being "derived from natural materials such as animals, plants, bacteria, and certain minerals". They are divided into three groups: biochemical substances, microbial pesticides, and plant-incorporated protectants (PIPs). Biochemical pesticides include substances of natural origin or synthetically derived equivalents that have exhibited minimal toxicity to humans and the environment. Microbial pesticides include microorganisms that control pests and demonstrate differentiated modes of action such as competition or inhibition. One of the best-known microbial biopesticides is Polyversum WP containing *Pythium oligandrum*, which demonstrates activity towards fungi including *Fusarium* sp., responsible for as much as 30–40% of the losses of economically important plants. Another example is Serenade ASO, a microbiological fungicide and bactericidal/static agent containing the *Bacillus subtilis* strain intended for the protection of various plants including fruits and vegetables. In turn, the PIPs are substances produced by genetically modified plants and the genetic material that has been added to the plant [8–10]. In the concept of sustainable agriculture, biofertilizers containing beneficial microorganisms also play an important role, helping to increase the resistance of plants and the rhizosphere to biotic and abiotic stresses. Ibáñez et al. [11], in a comprehensive review, underlined that a better-defined legal framework is necessary, both in the fields of biopesticides and biofertilizers, similar to those introduced in some countries, such as the USA.

As expected, the market for biopesticides will grow in the coming years; hence, the interest in searching for new compounds or microorganisms is very high. Among the microorganisms considered potential biopesticides, fermentative bacteria, such as lactic and propionic bacteria, deserve attention. These bacteria seem to be good candidates for biopesticides because they can inhibit the development of pathogens in crops. This is related to the ability of these microorganisms to produce metabolites such as organic acids (lactic, propionic, acetic, formic acids), hydrogen peroxide, diacetyl, bacteriocins and bacteriocin-like inhibitory substances (BLIS), and other metabolites such as phenyllactic acid, phenyl lactate, hydroxyphenyl lactate, cyclic dipeptides, 3-hydroxy fatty acids [12–17]. The exact mechanism of interaction between lactic and propionic bacteria is difficult to determine because usually it is not the result of a single metabolite but results from the interaction itself and possible synergism between different metabolites [12]. Moreover, antagonism between microorganisms may be related not only to the production of antimicrobial substances but also to competition for nutrients [15].

It should be emphasized that diseases occurring in crops generate large losses and pose a threat to human and animal health. Pathogenic fungi are one of the key factors responsible for their occurrence. One of the most devastating diseases of wheat is the common bunt, caused by *Tilletia caries* (syn. *Tilletia tritici*) and *Tilletia laevis* (syn. *Tilletia foetida*), which is observed worldwide. The effect of infection is the replacement of grains by bunt balls containing unpleasant-smelling brown to black spores. As a result, a reduction in grain yield and seed quality is observed [18,19]. In rye cultivars, a serious problem is stem smut caused by *Urocystis occulta*, a seed-borne disease that causes damage to the vegetative parts of plants including stems and leaves. Infected plants are usually darker green than normal and dwarfed. Although the disease is rarely responsible for severe losses in yield, periodically, its scale can be serious [20]. Among the most economically important genera of fungi in crops all over the world is *Fusarium*, responsible for Fusarium head blight (FHB)

and other diseases in cereals, such as seedling blight, root rot, and Fusarium crown rot. Moreover, it is worth emphasizing that *Fusarium* species produce mycotoxins, which may be harmful to humans and animals [21–23]. In potato production, the most destructive diseases are black scurf and stem canker, caused by the soil-borne fungus *Rhizoctonia solani*. This species is also pathogenic for other plants such as rice, maize, wheat, and lettuce [24,25]. Among the most ubiquitous fungi occurring in crops worldwide, *Alternaria* spp. is responsible for brown spot disease, one of the most destructive leaf spot diseases on a wide range of plants, including tobacco, tomato, citrus, and beans [26]. Among various species of *Alternaria*, the most frequently reported are *A. alternata*, commonly associated with vegetable brassicas, and *A. brassicae*, associated with rapeseed [27,28]. Fungal plant pathogens responsible for serious crop losses also include *Colletotrichum* spp. and *Sclerotinia* spp., which cause diseases in field crops, fruit, and vegetables. For example, fungi belonging to the *C. gloeosporioides* complex mainly affect the crown and cause crown rot; however, they can also be found on roots, stems, and fruit [29,30]. *Sclerotinia sclerotiorum* is a fungal pathogen that causes diseases in more than 400 hosts, including numerous fields, vegetables, fruits, ornamental crops, and other crops [31].

According to data found in the literature and to the best of our knowledge, LAB are known for their fungistatic properties; however, these findings are mainly from studies conducted in vitro. Data from mini-plot, field, or greenhouse experiments involving LAB are very limited since such studies are mainly conducted with microorganisms isolated from the rhizosphere, including the genera *Bacillus*, *Peanibacillus*, and *Pseudomonas*. Complex approaches are highly needed, including both in vitro and in vivo studies, to evaluate the potential of selected LAB as biological control agents. Therefore, the aim of the presented study was to comprehensively investigate the efficacy of the selected strains of LAB in the inhibition of fungi in laboratory research as well as in the control of the growth of some wheat and rye diseases (stem smut of rye, common bunt, and seedling blight) in mini-plot and field experiments, including the effect of bacteria on the growth of the plants and the yield.

2. Materials and Methods

2.1. Plant Material

Winter wheat variety Nadobna and winter rye variety Dańkowskie Złote were obtained from the Research Centre for Registration of Agrochemicals of the Institute of Plant Protection National Research Institute in Poznań, Poland.

2.2. Isolation of Lactic Acid Bacteria

LAB were isolated from corn silages prepared without any additional preservatives using mini-silo for research studies. The isolation process included homogenization of silage samples (10 g) in stomacher BagMixer Interscience with 90 mL of sterile saline solution, followed by serial dilution and cultivation on MRS agar (De Man Rogosa Sharpe Agar, Merck, Darmstadt, Germany). Incubation of the samples was carried out in an anaerobic atmosphere at 37 °C for 24 h. After incubation, clearly separated colonies were isolated and transferred onto an MRS liquid medium. Thus, obtained isolates were subjected to preliminary identification on the basis of morphology and phenotypic characteristics by microscopic evaluation and enzymatic tests. The selected isolates were Gram-positive catalase-negative rods and cocci. Ten selected LAB isolates were tested for their antifungal activity in the first stage of our research.

2.3. Preparation of Cell-Free Supernatant from Bacterial Cultures

Isolated strains were grown in MRS broth at 37 °C for 24 h. The bacterial population reached 10^8 CFU/mL. To obtain the cell-free supernatant (CFS), the bacterial cultures were centrifuged at 10,000 rpm for 5 min, the cells were removed, and the supernatant was filtered through a sterile membrane with a 0.22 µm pore size.

2.4. Antifungal Activity of Lactic Acid Bacteria

The antifungal activity of the tested LAB was determined against eight filamentous fungi. Four species of the genus *Fusarium* (*F. graminearum* KZF 1, *F. culmorum* KZF 5, *F. oxysporum* KZF 27, *F. poae* KZF 181) as well as *Alternaria alternata* KZF 32, *Rhizoctonia solani* KZF 38, *Colletotrichum gleosporoides* BPR 1303, and *Sclerotinia sclerotiorum* KZF 53 were obtained from the collection of the Research Centre for Registration of Agrochemicals, Institute of Plant Protection, and the Bank of Plant Pathogens and Investigation on Their Biodiversity, National Research Institute in Poznań, Poland. Data concerning the isolation source of indicator fungi are presented in Table S1 in the Supplementary Materials. The tested filamentous fungi were cultivated in Petri dishes (55 mm diameter) on potato dextrose agar (PDA, A&A Biotechnology, Poland) at 25 °C for 5–10 days. From the mature mycelia, the suspensions used to assess the antifungal activity of LAB were prepared as described below.

The antifungal activity of CFS was determined by broth microdilution. Aliquots of 100 µL of twofold dilutions of tested CFS from bacterial cultures at concentrations ranging from 0.4 to 50% were prepared in 96-well microtiter plates. The suspensions of microbial conidia and hyphae from homogenous fungal cultures were prepared in potato dextrose broth (A&A Biotechnology, Poland), mixed, and standardized to obtain a final cell concentration of 10^6 conidia/mL using a hemocytometer. Next, 100 µL of fungal suspension was introduced into the wells. The control was fungal culture without the addition of a CFS. After incubation, 100 µL of fungal culture from wells showing no visible growth as well as neighboring cells were spread on PDA medium and incubated for 5–10 days, depending on the indicator microorganisms. The results are expressed as the average of three replicates. The antifungal activity was expressed as the activity unit (AU) per milliliter of culture medium and quantified by taking the reciprocal of the highest dilution that exhibited a clear inhibition of fungi. The activity units were calculated using the following formula:

$$\text{AU mL}^{-1} = \frac{1000}{\text{d}}\,\text{D} \quad (1)$$

where

- d is the amount of supernatant per mL, and D is the dilution factor [32,33].

2.5. Identification of Lactic Acid Bacteria

Identification of the selected strains was carried out using the matrix-assisted laser desorption/ionization (MALDI) spectrometric technique (Microflex mass spectrometry, Bruker, Germany) according to the standard producers' protocol [34,35], using the data stored in Biotyper reference library of MALDI-Biotyper mass spectra and The National Center for Biotechnology Information (NCBI) for identification of obtained spectra. The interpretation of the results was based on Bruker MALDI-Biotyper criteria: for high confidence in results, the value of the identification index must score $\geq$2. A range from 1.99 to 1.70 indicates low-confidence identification, and a value lower than 1.70 equals lack of microorganism identification. The results were confirmed by *16S rRNA* sequencing. The sequences were obtained using primers 1492r (GGT TAC CTT GTT ACG ACT T) and S-D-Bact-0008 (AGA GTT TGA TCM TGG CTC AG) [36,37]. The 1500 base sequences were edited, combined, and generated using the GeneDoc 2.700. The PCR products were purified and sequenced by Genomed (Warsaw, Poland). Obtained sequences were analyzed using the Basic Local Alignment Search Tool (BLAST, Megablast algorithm), https://blast.ncbi.nlm.nih.gov/, (accessed on 21 August 2023) and submitted to GenBank. The unrooted phylogenetic tree was constructed by the neighbor-joining method to determine the closest LAB species using the MEGA X software [38].

2.6. Culture Preparation for Seed Treatment

LAB were propagated in MRS broth in Erlenmeyer flasks at 24 °C for 48 h. Next, the fresh culture of each strain was prepared in sterile MRS broth. The bacterial population in these cultures was maintained at 10^7–10^8 cells mL^{-1} by measuring optical density at 600 nm.

2.7. Seed Treatment

Before the mini-plot and field experiments, wheat and rye seeds were treated with microorganism cultures. Seeds from naturally infected plants collected in the previous season were additionally inoculated. The winter rye seeds were inoculated with *Urocystis occulta* by mixing 1 kg of seeds with 2 g of teliospores. The winter wheat seeds were inoculated with *Tilletia tritici* and *Tilletia foetida* by mixing 1 kg of grain with 2 g of sorus. After inoculation, seeds were treated with bacterial culture at the proportion of 300 mL of bacteria per 1 kg of seeds. Vibrance Gold 100 FS (active ingredients (a.i.) content: sedaxane 50 g/1 L + fludioxonil 25 g/L + difenoconazole 25 g/L; dose: 200 mL + 400 mL of water/100 kg of seeds) and Vitavax 200 FS (active ingredients (a.i.) content: carboxin 200 g/1 L + thiram 200 g/L; dose: 300 mL + 300 mL of water/100 kg of seeds) registered in Poland for the protection of cereals against the diseases studied in this work were used as standard and comparative seed treatments. Vibrance Gold 100 FS was used at the proportion of 2 mL mixed with 4 mL of water per 1 kg of seeds, while Vitavax 200 FS was used at the proportion of 3 mL mixed with 3 mL of water per 1 kg of seeds. The application of seed treatment was carried out in a seed dresser HEGE 11 with a tank volume of 14.5 L.

2.8. Mini-Plot Experiments

Mini-plot experiments were conducted during the growing of seasons of 2015/2016 and 2016/2017 at the Institute of Plant Protection, National Research Institute in Poznań. (Global Positioning System (GPS) coordinates: N 52°23′48.471″, E 16°51′20.585″). Treated seeds were sown in 28 cm diameter plots with 5 g seeds each (approx. 100 seeds). The experiments were performed in four replicates. In the mini-plot experiments, the efficacy of tested bacteria on the occurrence and severity of symptoms of some diseases was determined as follows. Stem smut of rye caused by *Urocystis occulta* was evaluated by counting infected stems in the entire mini-plot, and the results were converted to the average percentage of infected stems in the mini-plot. Common bunt caused by mixed infection with *Tilletia tritici* and *Tilletia foetida* in wheat was determined by counting infected ears in the entire mini-plot and expressed as the average percentage of infected ears in the mini-plot. All stems (rye) and ears (wheat) in each mini-plot were counted, and then infected plants were separated (according to the parameters of the disease specified in Section 2.9.1. for the common bunt and stem smut of rye). On this basis, the percentage of infected plants was determined. In each experiment, Vitavax FS 200 and Vibrance Gold 100 FS were used as positive controls, depending on the plant, while untreated samples were used as negative controls.

2.9. Field Experiments

Field trials were conducted during the growing seasons of 2015/2016 and 2016/2017 at the Field Experimental Station of the Institute of Plant Protection, National Research Institute in Winna Góra (GPS coordinates: N 52°12′41.7″, E 17°25′45.6″) using the system of randomly completed blocks in four repetitions. The area of each plot was 16.5 m^2. Standard herbicidal and insecticidal applications were conducted without using fungicides (Tables S2 and S3 in the Supplementary Materials). A Wintersteiger plot seeder (Wintersteiger, Ried, Austria) with a row spacing of 12.5 cm was used to sow the seeds. The investigation was conducted in accordance with European and Mediterranean Plant Protection Organization (EPPO) Guideline Nos. PP 1/181 (4) [39], PP 1/152 (4) [40], PP 1/135 (4) [41], PP 1/19 (4) [42]. Meteorological conditions regarding air temperature and precipitation were collected by the meteorological station located in Winna Góra and presented

in Tables S4 and S5 in the Supplementary Materials. In the field experiments, the efficacy of tested bacteria on the occurrence and severity of symptoms of some diseases as well as crop characteristics, including plant emergence, phytotoxicity of tested treatments, yield, and weight of 1000 seeds, were determined. In each experiment, Vitavax FS 200 and Vibrance Gold 100 FS were used as positive controls, depending on the plant, while untreated samples were used as negative controls.

2.9.1. Disease Assessment

The efficacy of tested bacteria on the occurrence and severity of symptoms of some diseases was determined as follows. Seedling blight, caused by various fungi, including *Fusarium* spp., was assessed both in wheat and rye cultivation as the percentage of plants with disease symptoms and was calculated in an evaluated sample taken from four segments of rows 0.25 m long each. Evaluations were performed using Biologische Bundesanstalt, Bundessortenamt und Chemical Industry (BBCH) uniform coding system of the phenological development stages of plants [43]. The assessments were carried out on 20 November 2015 (BBCH 12) and 12 December 2016 (BBCH 12) in the fields of wheat as well as on 20 November 2015 (BBCH 13) and 12 December 2016 (BBCH 12) in the field of rye. Common bunt caused by mixed infection with *Tilletia tritici* and *Tilletia foetida* in the wheat fields was determined as the percentage of infected ears in an evaluated sample of fifty ears per plot. The assessments have been were carried out on 8 July 2016 (BBCH 87) and 16 July 2017 (BBCH 87). The following parameters of the disease were determined: in seedling blight, brown spots and necrosis on lower leaf sheaths; in common bunt, sori (diseased kernels in ears) containing dark masses of teliospores; in stem smut of rye, characteristic long dark streaks on stems and leaves containing masses of teliospores. Stem smut of rye caused by *Urocystis occulta* was evaluated in the rye crops by counting the infected stems on the whole plot area.

2.9.2. Plant Emergence and Overwintering Determination

Plant emergence was calculated by counting the number of emerged plants on each plot in 10 segments of rows 1 m long each. The result was converted into the average number of plants per 1 m^2. In the spring, overwintering was assessed in the sections marked in autumn. The emergence was assessed in BBCH 12 phase, while overwintering was assessed in BBCH 23 phase.

2.9.3. Assessment of the Phytotoxicity Effects of Bacterial Cultures on the Crop

Phytotoxicity assessment was performed in accordance with EPPO standard PP1/135 (4) [41]. The evaluation of phytotoxicity effects of bacterial cultures and standard seed treatment applied to crops was performed visually by comparing the condition of the plants in the plots treated with fungicide to untreated plots. The intensity of damage to the plant was expressed in percentage (0%: no symptoms of phytotoxic effects, 100%: total destruction).

2.9.4. Yield Grain Determination

Grain was harvested with a plot harvester Wintersteiger, model Classic (Ried, Austria) to quantify the grain yield. The weight of grain and the humidity from each harvested plot were evaluated using a Foss Infratec 1241 analyzer. The grain yield results were expressed as tons per hectare, assuming a standard moisture content of 14%.

2.9.5. Weight of 1000 Grains Assessment

Randomly selected samples of grain were collected. Each sample was divided into three batches of 200 grains and weighed. The results are presented as the average weight of 1000 grains in grams.

2.10. Statistical Analysis

The results of the experiments were presented as the mean of parallel repetitions and subjected to the analysis of variance, and for the comparison of the significance of data obtained, the Student's *t* test was used, setting the least significant difference (LSD) at a significance level of 5%. The Agriculture Research Manager program, version 9, was used for statistical analysis.

3. Results

3.1. Antifungal Activity of Isolated Lactic Acid Bacteria

Ten isolates of bacteria preliminarily identified as LAB based on their morphological (Gram-positive rods) and phenotypical (catalase-negative bacteria) features were used to determine their antifungal activity towards eight different filamentous fungi. The results are presented in Table 1 and Figures S1–S8 in the Supplementary Materials. The results showed that all tested LAB isolates exhibited activity towards the tested fungi. The activity was dependent mainly on the fungal species. *Rhizoctonia solani* KZF 38 was the most susceptible fungus, where CFS obtained from five LAB isolates (P7.Z, P7L, P7, A2.P, and P41) demonstrated activity reaching 1600 AU/mL. LAB also strongly inhibited *A. alternata* KZF 32, *C. gleosporioides* BPR 1303, and *S. sclerotiorum* KZF 53. The AU/mL value noted towards the above-mentioned strains ranged from 400 to 800 except for isolate P42, which showed the lowest antifungal activity against all tested fungi. *Fusarium* species were also sensitive to the LAB metabolites; however, the activity usually did not exceed 400 AU/mL. Only two LAB isolates, G13 and S12, inhibited one of the *Fusarium* species, *F. graminearum*, at a level of 800 AU/mL. Based on the obtained results, five isolates (marked in gray in Table 1) were selected for further experiments, including mini-plot and field experiments as well as identification.

Table 1. Antifungal activity of LAB isolates expressed in AU/mL.

LAB Strain	Antifungal Activity (AU/mL)							
	Fusarium graminearum	***Fusarium culmorum***	***Fusarium oxysporum***	***Fusarium poae***	***Rhizoctonia solani***	***Alternaria alternata***	***Colletotrichum gleosporioides***	***Sclerotinia sclerotiorum***
P7.Z	400	400	200	400	1600	800	800	800
P7L	400	400	200	400	1600	800	800	800
P7	400	400	400	400	1600	800	800	800
A2.P	400	400	200	400	1600	400	400	400
P40	400	400	200	400	400	800	800	800
P41	400	400	400	400	1600	1600	800	800
P42	<200	<200	<200	<200	400	<200	200	<200
G13	800	400	200	400	800	400	800	800
S12	800	400	400	400	800	400	400	400
A7	400	400	400	400	800	400	400	800

3.2. The Identification of Selected Strains of Lactic Acid Bacteria

Five selected LAB isolates were identified by MALDI-Biotyper mass spectrometry and sequencing of the *16S rRNA* gene. Results covering the LAB genus and identification value are compiled in Table S6 in the Supplementary Materials. Among five isolates, one (A7) belonged to the *Lactiplantibacillus plantarum* species, two (G13 and S12) were identified as *Lacticaseibacillus paracasei*, and two (P7 and P41) as *Lentilactobacillus buchneri*. The query coverage of the presented LAB identification results obtained using BLAST was 99–100%. The combined partial sequences of isolates (Table S7 in the Supplementary Materials) were deposited in GenBank. Following the phylogenetic analysis (Figure 1), LAB strains P7 and P41 were placed in the cluster making up the *Lentilactobacillus* cluster, *L. buchneri* subgroup. The strain A7 was placed in the *Lactiplantibacillus* group, subgroup *L. plantarum*. The LAB strains G13 and S12 were positioned in the *Lacticaseibacillus* genus, subgroup *L. paracasei*.

The conducted phylogenetic analysis confirmed that the results of the isolated LAB strain identification were correct.

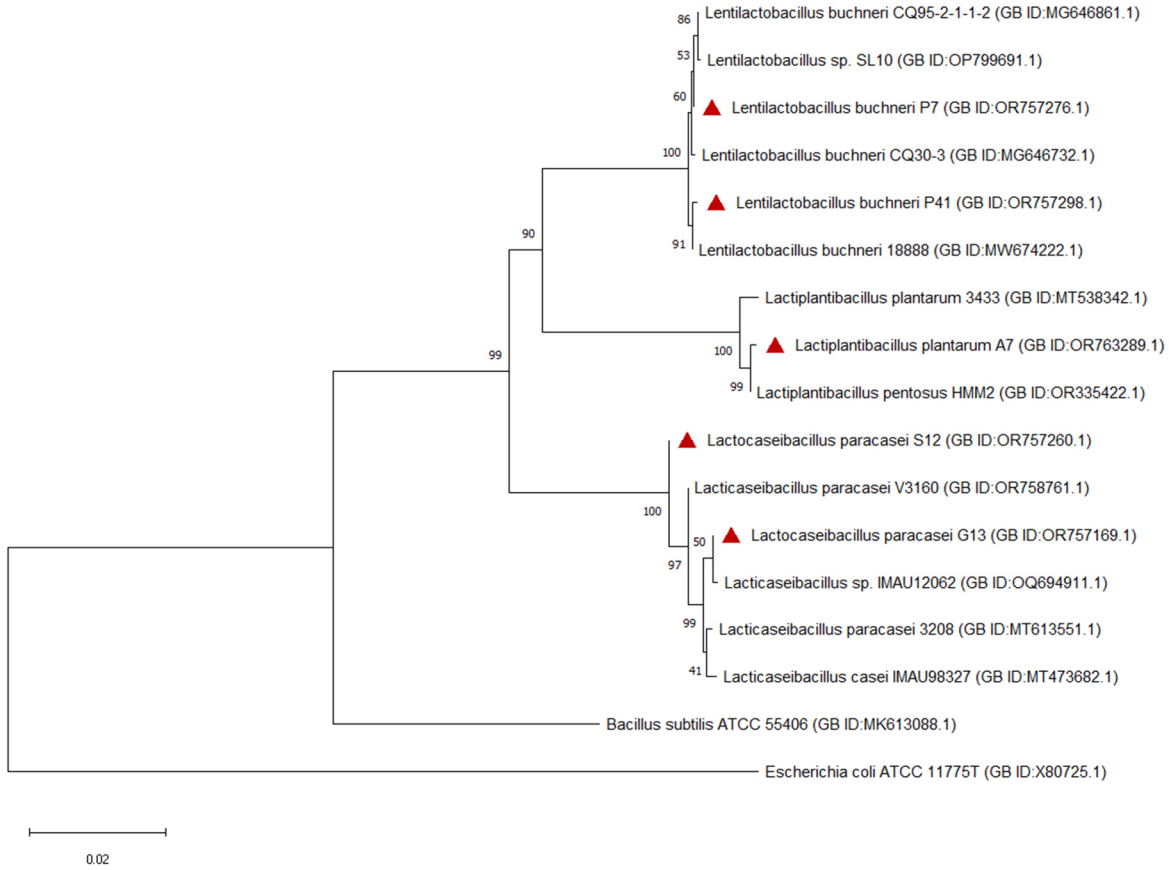

Figure 1. Phylogenetic tree based on *16S rRNA* gene sequences showing the position of selected LAB isolates (marked with a filled red triangle pointing upwards). *Bacillus subtilis* ATCC 55406 and *E. coli* ATCC 11775T were taken as an out-group. Bootstrap values are given at branching points.

3.3. The Efficacy of Tested Bacteria in the Control of Chosen Diseases of Wheat and Rye in Mini-Plot Experiments

The efficacy of selected LAB strains in the control of stem smut of rye and common bunt of wheat is presented in Tables 2 and 3. The studies were conducted for two consecutive years, and the disease severity was determined by evaluation of the percentage of infected stems (in rye) or ears (in wheat), as described in Section 2. The results showed high activity of LAB strains in the control of fungal diseases; however, the effectiveness in reducing disease symptoms depended on the bacterial strain and year of the experiment. All tested LAB strains significantly decreased the percentage of infected stems caused by *Urocystis occulta* (Table 2). It is worth mentioning that differences were not observed between the treatment with LAB and the standard seed treatment (Vibrance Gold 100 FS except for *L. buchneri* P41 in the first season and *L. paracasei* G13 in the second season). The highest efficacy among the tested bacteria was seen in *L. plantarum* A7 and *L. paracasei* S12, with about 90% efficacy in both years of the experiment. In the first year of the study, *L. paracasei* G13 also demonstrated high effectiveness in the control of stem smut in rye.

Table 2. The efficacy of LAB towards stem smut of rye (*Urocystis occulta*) in mini-plot experiments.

Treatment	Season 1		Season 2	
	Average % of Infected Stems	Average % of Efficacy	Average % of Infected Stems	Average % of Efficacy
Control/Untreated	11.3 [a]	-	11.9 [a]	-
Lactiplantibacillus plantarum A7	0.6 [b]	94.7	1.3 [b]	89.1
Lacticaseibacillus paracasei G13	0.6 [b]	94.7	7.8 [a]	34.5
Lacticaseibacillus paracasei S12	1.1 [b]	90.3	1.1 [b]	90.8
Lentilactobacillus buchneri P7	2.5 [b]	77.9	1.5 [b]	87.4
Lentilactobacillus buchneri P41	5.2 [ab]	54.0	2.1 [b]	82.4
Vibrance Gold 100 FS	0.6 [b]	94.7	0.4 [b]	96.6
LSD 0.05	7.06		4.46	

a,b—values in columns followed by the same letter do not differ significantly at $p = 0.05$.

Table 3. The efficacy of LAB towards common bunt (mixed infection with *Tilletia tritici* and *Tilletia foetida*) in mini-plot experiments.

Treatment	Season 1		Season 2	
	Average % of Infected Ears	Average % of Efficacy	Average % of Infected Ears	Average % of Efficacy
Control/Untreated	57.5 [a]	-	73.9 [a]	-
Lactiplantibacillus plantarum A7	28.7 [bc]	50.1	0.3 [c]	99.6
Lacticaseibacillus paracasei G13	15.0 [c]	73.9	55.6 [b]	24.8
Lacticaseibacillus paracasei S12	36.6 [b]	36.3	1.9 [c]	97.4
Lentilactobacillus buchneri P7	27.3 [bc]	52.5	1.0 [c]	98.6
Lentilactobacillus buchneri P41	32.7 [b]	43.1	5.2 [c]	93.0
Vitavax 200 FS	0.0 [d]	100	1.4 [c]	98.1
LSD 0.05	14.49		8.35	

a–d—values in columns followed by the same letter do not differ significantly at $p = 0.05$.

The various results of the impact of the LAB strains on the development of the common bunt depended on the year of the study (Table 3). In the first year of the experiment, the efficacy of tested bacteria in disease control differed significantly and ranged from 36.3% for *L. paracasei* S12 to 73.9% for *L. paracasei* G13, while in the second year, the effectiveness was much higher for the majority of strains, except for *L. paracasei* G13. It reached up to 99.6% for *L. plantarum* A7. Both strains of *L. buchneri* as well as *L. paracasei* S12 also demonstrated more than 90% activity. It should be noted that all tested bacteria significantly limited the symptoms of the disease compared to the control trials in both years of experiments. There were also significant differences between trials treated with bacteria and Vitavax 200 FS during the first season, while in the second year, the difference was noted only in the case of *L. paracasei* G13 when compared to the standard product.

3.4. The Efficacy of Tested Bacteria in the Control of Chosen Diseases of Wheat and Rye in Field Experiments

3.4.1. The Effect of Tested Bacteria on the Fungal Disease Severity

The field experiments were conducted simultaneously to research in the mini-plots, but they covered a much wider range of observations, including the evaluation of the efficacy of tested LAB strains in the control of stem smut in rye, common bunt in wheat, seedling blight, as well as yield, phytotoxicity, and emergence of rye and wheat. Data provided in Tables S4 and S5 from the Supplementary Materials indicate that the temperature distribution was similar in both research seasons; however, in season 1, the average temperature was higher in the periods November–December and February–March. Minus temperatures were recorded only in January (in the first weeks of the month in 2016 and all weeks in

2017). Average humidity in the season of 2015/2016 remained at a relatively constant level (68.66–88.25%), with the lowest values falling in the period of April–June 2016. For most of the 2015/2016 season, the total rainfall was ≤36 mm, and in July only did it reach a level of 115.90 mm (a typical season of intense rainfall in Poland). During the second season, there was humidity in the period October 2016–January 2017. Until the middle of the second research season, the amount of total rainfall was lower than in the first season, but in the second half it was much higher, especially in the period April–August. Due to different weather conditions, the severity of diseases varied between seasons.

The first disease observed in the field experiment was seedling blight, which was not evaluated on mini-plots due to the limited number of plants (the evaluation of seedling blight requires removing the whole plant from the soil and making observations of the root neck). Seedling blight occurred in both seasons and in both crops, however, at different severities. The efficacy of LAB in inhibiting this disease in the rye was rather low and did not exceed 50.3% (Table 4). In the first year of the experiment, only the standard seed treatment Vibrance Gold 100 FS significantly reduced the occurrence of the disease, while the effectiveness of LAB was rather weak and ranged from 12.8 to 39.1%, with the best results demonstrated by *L. plantarum* A7. In the second year of the experiment, *L. plantarum* A7 as well as both strains of *L. paracasei*, G13 and S12, significantly limited the disease development, and their efficiency ranged from 36% for *L. paracasei* G13 to 50.3% for *L. paracasei* S12.

Table 4. The efficacy of LAB towards seedling blight (caused by *Fusarium* spp.) in rye and wheat in the field experiments.

Treatment	Rye				Wheat			
	Season 1		Season 2		Season 1		Season 2	
	Average % of Infected Plants	Average % of Efficacy	Average % of Infected Plants	Average % of Efficacy	Average % of Infected Plants	Average % of Efficacy	Average % of Infected Plants	Average % of Efficacy
Control/Untreated	25.8 ^{a}	-	28.6 ^{a}	-	43.5 ^{a}	-	23.9 ^{a}	-
L. plantarum A7	15.7 ^{ab}	39.1	16.0 ^{bc}	44.1	21.6 ^{b}	50.3	14.1 ^{ab}	41.0
L. paracasei G13	25.7 ^{a}	0.4	18.3 ^{b}	36.0	22.0 ^{b}	49.4	12.6 ^{b}	47.3
L. paracasei S12	22.5 ^{a}	12.8	14.2 ^{bc}	50.3	22.4 ^{b}	48.5	15.4 ^{a}	35.6
L. buchneri P7	21.6 ^{a}	16.3	22.9 ^{a}	19.9	27.3 ^{b}	37.2	7.7 ^{b}	67.8
L. buchneri P41	22.5 ^{a}	12.8	20.4 ^{ab}	28.7	23.2 ^{b}	46.7	12.6 ^{a}	47.3
Vibrance Gold 100 FS	11.3 ^{b}	56.2	7.2 ^{c}	74.8	nt.	nt.	nt.	nt.
Vitavax 100 FS	nt.	nt.	nt.	nt.	18.7 ^{b}	57.0	8.0 ^{a}	66.5
LSD 0.05	10.68		9.52		10.18		9.82	

nt.: not tested. a–c—values in columns followed by the same letter do not differ significantly at $p = 0.05$.

The effectiveness of LAB in limiting seedling blight in wheat was higher than in rye (Table 4). Generally, the treatment of seeds with LAB cultures caused a reduction in disease symptoms. In the first season, all tested bacteria significantly reduced the seedling blight symptoms, and the average efficacy ranged from 37.2 for *L. buchneri* P7 to 50.3% for *L. plantarum* A7. Both strains of *L. paracasei* and *L. buchneri* P41 demonstrated nearly 50% efficacy. During the second year of the experiment, only *L. buchneri* P7 showed efficacy at a level similar to standard seed treatment, with an average percentage of infected plants significantly different than control trials. The effectiveness of other strains was lower and was significantly different from the trials treated with standard seed treatment. It can be emphasized that the highest efficacy in both seasons was demonstrated by the standard seed treatment Vitavax 200 FS.

During the field experiment, the efficacy of LAB strains in the control of stem smut rye and common bunt was also determined, similar to the mini-plots trials. According to the results presented in Table 5, it could be stated that the efficacy of LAB in inhibiting the growth of *Urocystis occulta* was very high and significantly different than the control trial. The differences between standard seed treatment and trials treated with LAB cultures were not observed. The effectiveness of limiting the disease symptoms by LAB strains

ranged from 88.5 to 100% in the first year of the experiment and from 95.6 to 99.5% in the second year. The standard seed treatment Vibrance Gold 100 FS totally inhibited the disease development. Whereas, for the LAB strains, the best results were observed for *L. plantarum* A7 and *L. paracasei* G13. High effectiveness was also demonstrated by *L. paracasei* S12, while *L. buchneri* P7 and P41 showed lower efficacy in the first season and higher effectiveness in the second year. However, it is worth noting that, during the first season, the plant infestation was much higher than the following year.

Table 5. The efficacy of LAB towards stem smut of rye (*Urocystis occulta*) in the field experiments.

Treatment	Season 1		Season 2	
	Average Number of Infected Stems per Plot	Average % of Efficacy	Average Number of Infected Stems per Plot	Average % of Efficacy
Control/Untreated	503.3 ^{a}	-	91.8 ^{a}	-
Lactiplantibacillus plantarum A7	0.0 ^{b}	100	0.5 ^{b}	99.5
Lacticaseibacillus paracasei G13	16.5 ^{b}	96.7	1.8 ^{b}	98.0
Lacticaseibacillus paracasei S12	24.8 ^{b}	95.1	4.0 ^{b}	95.6
Lentilactobacillus buchneri P7	57.8 ^{b}	88.5	0.0 ^{b}	100
Lentilactobacillus buchneri P41	66.0 ^{b}	86.9	1.3 ^{b}	98.6
Vibrance Gold 100 FS	0.0 ^{b}	100	0.0 ^{b}	100
LSD 0.05	125.68		19.50	

a,b—values in columns followed by the same letter do not differ significantly at $p = 0.05$.

Common bunt occurred at quite low intensity (17.5% of infected ears) in the first year of the study and at a much higher intensity (56.5%) in the following season (Table 6). The impact of bacteria applied as a seed treatment was more variable than that of the stem smut rye. The results in Table 6 show that tested LAB limited the disease development; however, their effectiveness depended on the strain and season of study. Similar to the results obtained for stem smut rye control, *L. plantarum* A7 demonstrated the highest efficacy in the control of common bunt, reaching 97.1 and 90.3% efficacy, depending on the year. The results demonstrated by other strains were much more varied. *L. buchneri* P7 showed high efficacy in the second season (99.1%), with much lower effectiveness observed during the first year of the experiment. Similarly, *L. paracasei* G13 in the first season demonstrated 91.4% efficacy; however, in the next year, the efficiency was 44.2% and did not differ from the control trial. As the results showed, the standard seed treatment Vitavax 200 FS totally inhibited the disease development.

Table 6. The efficacy of LAB towards common bunt (mixed infection with *Tilletia tritici* and *Tilletia foetida*) in the field experiments.

Treatment	Season 1		Season 2	
	Average % of Infected Ears	Average % of Efficacy	Average % of Infected Ears	Average % of Efficacy
Control/Untreated	17.5 ^{a}	-	56.5 ^{a}	-
Lactiplantibacillus plantarum A7	0.5 ^{d}	97.1	5.5 ^{b}	90.3
Lacticaseibacillus paracasei G13	1.5 ^{c}	91.4	31.5 ^{ab}	44.2
Lacticaseibacillus paracasei S12	2.0 ^{c}	88.6	19.5 ^{b}	65.5
Lentilactobacillus buchneri P7	4.0 ^{c}	77.1	0.5 ^{b}	99.1
Lentilactobacillus buchneri P41	6.5 ^{bc}	62.9	7.0 ^{b}	87.6
Vitavax 200 FS	0.0 ^{d}	100	0.0 ^{b}	100
LSD 0.05	5.11		25.29	

a–d—values in columns followed by the same letter do not differ significantly at $p = 0.05$.

3.4.2. The Effect of LAB on Plant Growth, Yield Parameters, and Phytotoxicity

During field tests, the influence of the selected LAB used for seed treatment on the emergence and overwintering of wheat and rye was tested (Tables 7 and 8). Furthermore, crop yield parameters (Tables 9 and 10) as well as phytotoxicity (Table S8 in the Supplementary Materials) were also determined.

Table 7. The effect of LAB treatment on the emergence of wheat.

Treatment	Season 1		Season 2	
	Plant Emergence *	Overwintering *	Plant Emergence *	Overwintering *
	12 November 2015 BBCH 12	17 March 2016 BBCH 23	12 December 2016 BBCH 12	31 March 2017 BBCH 23
Control/Untreated	94.00 ^{a}	66.75 ^{a}	55.00 ^{a}	52.88 ^{a}
Lactiplantibacillus plantarum A7	85.00 ^{ab}	74.00 ^{a}	44.38 ^{a}	43.00 ^{a}
Lacticaseibacillus paracasei G13	76.75 ^{ab}	65.25 ^{a}	46.63 ^{a}	45.00 ^{a}
Lacticaseibacillus paracasei S12	87.50 ^{ab}	70.25 ^{a}	47.00 ^{a}	44.75 ^{a}
Lentilactobacillus buchneri P7	75.25 ^{b}	57.75 ^{a}	49.50 ^{a}	46.25 ^{a}
Lentilactobacillus buchneri P41	82.00 ^{ab}	65.75 ^{a}	46.63 ^{a}	44.63 ^{a}
Vitavax 200 FS	95.75 ^{a}	77.50 ^{a}	49.50 ^{a}	47.00 ^{a}
LSD 0.05	18.382	16.557	11.039	11.256

* The average number of plants per 1 m. a,b—values in columns followed by the same letter do not differ significantly at $p = 0.05$.

Table 8. The effect of LAB treatment on the emergence of rye.

Treatment.	Season 1		Season 2	
	Plant Emergence *	Overwintering *	Plant Emergence *	Overwintering *
	12 November 2015 BBCH 12	17 March 2016 BBCH 23	12 December 2016 BBCH 12	31 March 2017 BBCH 23
Control/Untreated	80.50 ^{a}	66.50 ^{a}	44.13 ^{a}	42.38 ^{a}
Lactiplantibacillus plantarum A7	59.25 ^{b}	52.00 ^{ab}	30.25 ^{b}	28.38 ^{c}
Lacticaseibacillus paracasei G13	50.75 ^{b}	45.50 ^{b}	44.00 ^{a}	41.00 ^{ab}
Lacticaseibacillus paracasei S12	45.75 ^{b}	37.50 ^{b}	33.50 ^{b}	29.88 ^{c}
Lentilactobacillus buchneri P7	58.00 ^{b}	54.00 ^{a}	29.00 ^{b}	27.50 ^{c}
Lentilactobacillus buchneri P41	65.25 ^{ab}	54.75 ^{a}	34.75 ^{b}	32.00 ^{bc}
Vibrance Gold FS	83.75 ^{a}	74.75 ^{a}	50.63 ^{a}	48.25 ^{a}
LSD 0.05	16.583	15.041	9.350	10.077

* The average number of plants per 1 m. a–c—values in columns followed by the same letter do not differ significantly at $p = 0.05$.

The results in Table 7 showed that the treatment of wheat seeds with LAB did not influence plant emergence or overwintering. In the wheat crop, some tendency to decrease plant emergence was observed; however, the differences were not significant. However, the plant emergence as well as the overwintering were significantly higher in the first year of the tests than in the second in all samples treated with LAB.

In rye crops, a significant decrease in plant emergence was observed (Table 8) in both seasons and in all tested LAB except for *L. paracasei* G13, which did not influence this parameter. The overwintering of plants was significantly reduced in trials with *L. plantarum* A7 and both *L. buchneri* strains during the first season of the experiment, while during the second season, a significant decrease in overwintering was observed only in trials with *L. paracasei* G13. However, it can be noted that a lower number of overwintered plants was observed in all trials with LAB, while in trials with standard seed treatment Vibrance Gold 100 FS, there was a tendency to increase the overwintering, although significant differences were not observed.

Table 9. The effect of LAB treatment on rye yield.

Treatment	Grain Yield				Weight of 1000 Grains			
	Season 1		Season 2		Season 1		Season 2	
	t/ha	% Increase *	t/ha	% Increase *	g	% Increase *	g	% Increase *
Control/Untreated	6.43 ^{b}	-	5.63 ^{c}	-	33.31 ^{b}	-	36.35 ^{b}	-
L. plantarum A7	6.30 ^{c}	−1.95	5.88 ^{c}	4.44	36.21 ^{a}	8.71	35.43 ^{b}	−2.53
L. paracasei G13	6.75 ^{a}	5.06	5.78 ^{c}	2.67	35.93 ^{a}	7.87	36.45 ^{ab}	0.27
L. paracasei S12	6.43 ^{b}	0.00	5.60 ^{c}	−0.44	36.69 ^{a}	10.16	37.82 ^{a}	4.04
L. buchneri P7	6.28 ^{c}	−2.33	5.78 ^{c}	2.67	37.07 ^{a}	11.28	38.81 ^{a}	6.75
L. buchneri P41	6.23 ^{c}	−3.11	6.75 ^{a}	20.00	33.30 ^{b}	−0.03	38.96 ^{a}	7.16
Vibrance Gold 100 FS	6.18 ^{c}	−3.89	6.38 ^{b}	13.33	33.12 ^{b}	−0.56	37.02 ^{a}	1.83
LSD 0.05	0.122		0.282		2.043		2.529	

* increase with different treatments calculated relatively to control/untreated. a–c—values in columns followed by the same letter do not differ significantly at $p = 0.05$.

Table 10. The effect of LAB treatment on wheat yield.

Treatment	Grain Yield				Weight of 1000 Grains			
	Season 1		Season 2		Season 1		Season 2	
	t/ha	% Increase *	t/ha	% Increase *	g	% Increase *	g	% Increase *
Control/Untreated	1.15 ^{e}	-	2.28 ^{d}	-	34.61 ^{b}	-	35.23 ^{a}	-
L. plantarum A7	3.00 ^{b}	160.87	5.40 ^{a}	137.36	37.12 ^{ab}	7.26	36.61 ^{a}	3.94
L. paracasei G13	3.40 ^{a}	195.65	3.25 ^{c}	42.86	38.31 ^{ab}	10.68	35.93 ^{a}	2.01
L. paracasei S12	2.50 ^{c}	117.39	5.13 ^{a}	125.27	43.43 ^{a}	25.48	38.23 ^{a}	8.53
L. buchneri P7	2.53 ^{c}	119.57	5.25 ^{a}	130.77	35.82 ^{b}	3.51	35.50 ^{a}	0.78
L. buchneri P41	2.20 ^{d}	91.30	4.78 ^{b}	109.89	35.10 ^{b}	1.41	35.60 ^{a}	1.07
Vitavax 200 FS	3.50 ^{a}	204.35	5.20 ^{a}	128.57	38.86 ^{ab}	12.27	36.26 ^{a}	2.94
LSD 0.05	0.216		0.309		7.911		3.516	

* increase with different treatments calculated relatively to control/untreated. a–e—values in columns followed by the same letter do not differ significantly at $p = 0.05$.

The treatment of rye seeds with LAB had varied effects on yield (Table 9). In the first season, a significant decrease in grain yield was observed in the trials with *L. plantarum* A7 and both strains of *L. buchneri* as well as the standard seed treatment Vibrance Gold 200 FS, while in the trials with *L. paracasei* G13, an increase in grain yield was noticed. In turn, in the next year of the experiment, *L. buchneri* P41 and the standard seed treatment caused an increased grain yield, while the other strains had no effect on yield. An effect of treatment with LAB on the weight of 1000 grains was also observed. A significant increase in this parameter was noticed in the trials treated with *L. plantarum* A7, both strains of *L. paracasei* and *L. buchneri* P7, as well as the standard seed treatment in the first season of the experiment. In the next year of research, only in the trials with *L. buchneri* P41 was there a significant increase in the weight of 1000 grains, while other LAB strains were not influenced by this parameter.

Treatment of wheat seeds with LAB had a positive effect on yield (Table 10) during both seasons of field experiments. In all fields with LAB as well as trials treated with the standard seed treatment, a significant increase in grain yield was noted. The greatest differences were observed in the trials treated with *L. plantarum* A7 in both seasons, *L. paracasei* G13 during the first season, and *L. buchneri* P7 during the second season of the experiment compared to the control trial. It is noteworthy that the best results obtained with LAB treatment were comparable to results for trials treated with the seed treatment Vibrance Gold 100 FS. However, no differences were found in the weight of 1000 grains between the samples treated with LAB and standard treatments and control trials. The trials treated with *L. paracasei* S12, which caused a significant increase in grain yield, were exceptions.

During the field experiments, at specific growth stages of the plants, the phytotoxicity of LAB treatment was determined. As shown in Table S7 in the Supplementary Materials, no phytotoxicity was found in any of the trials.

4. Discussion

The intensification of production in the agricultural sector and increased usage of chemical pesticides and fertilizers strongly affect the ecological balance in the environment, causing soil and water pollution. Therefore, the concept of sustainable agriculture is gaining increased attention. According to the FAO definition, sustainable agriculture emphasizes the environmentally friendly production of healthy and high-quality food with care for animal welfare and biodiversity protection, drawing attention also to ensuring income for farmers. To meet the challenge of farming sustainability, various models of agricultural production, including integrated farming systems (IFS) or low-input sustainable agriculture (LISA) programs, as well as alternative agriculture systems such as organic, biodynamic, low external input or resource-conserving, and regenerative, have been developed [44]. An integral part of this concept is the biological control of plant pathogens, including the use of microorganisms and natural compounds such as essential oils. Among the microorganisms used as biocontrol agents, groups of plant-promoting rhizobacteria [45–47] and fungi [48,49] are being intensively researched. However, in recent years, an increase in the use of LAB as antifungal agents has been observed [50]. These bacteria are ubiquitous members of different plant microbiomes, but in soil, their presence is rather minimal; therefore, they have not often been the subject of tests in field research, and data in this area are very limited.

In this paper, we have presented a wide range of research on the possibility of using LAB as potential plant protection agents, starting from the isolation of LAB from silages and their in vitro characterization in terms of antifungal properties to their use under in vivo conditions, first in mini-plots and then in the field. Many authors have described the antifungal properties of LAB, indicating the activity of these bacteria towards filamentous fungi from genera *Aspergillus*, *Penicillium*, *Cladosporium*, *Fusarium*, *Sclerotinia*, *Alternaria,* and *Rhizoctonia,* which pose a serious problem in food chains, since they cause diseases in plants and influence the safety and quality of food and feed [51–55]. Our study confirms the capacity of LAB to inhibit the growth of some plant pathogenic fungi. Ten strains of LAB isolates from silages demonstrated antifungal activity towards eight species of fungi belonging to the *Fusarium*, *Sclerotinia*, *Colletotrichum,* and *Rhizoctonia* genera. The activity of LAB depended on the bacterial strain and fungal species, with *R. solani* being the most susceptible fungus, while *Fusarium* sp. turned out to be much less sensitive. Similar observations have been made by other authors. Magnusson et al. [51] screened more than 1200 isolates of LAB from different environments. Approximately 10% of isolates demonstrated antifungal activity, and some of them strongly inhibited the growth of *Aspergillus fumigatus*, *A. nidulans*, *Penicillium commune*, *Fusarium sporotrichioides*, and the yeast *Rhodotorula mucilaginosa*. The authors observed different activity against *Penicillium commune*, *Aspergillus fumigatus*, *A. nidulans,* and *Fusarium sporotrichioides*, while *P. roqueforti* was not inhibited and *Rhodotorula mucilaginosa* was inhibited only by some of the isolates. As the authors found, the results depended mainly on the fungal strain but also on the LAB strain. Dalie et al. [56] screened 67 isolates of the LAB against *Fusarium proliferatum* and *F. verticillioides* using the overlay method and observed different activities of bacteria tested with *Pediococcus pentosaceus* (L006) as the most efficient strain. Among different LAB species, one of the most well-known species with antifungal activity is *L. plantarum*, which was also one of the strains used in this study. Russo et al. [57] showed antifungal properties of 88 *L. plantarum* strains against fungi belonging to *Aspergillus*, *Penicillium*, *Fusarium,* and *Cladosporium* genera, emphasizing that some fungal species (*A. flavus*, *P. roqueforti*, *Cladosporium* spp., and *A. niger*) demonstrated high resistance towards the antifungal metabolites of LAB. Similarly, Steglińska et al. [58] selected *Lactiplantibacillus plantarum* KB2 LAB 03 as the most effective agent after screening 100 LAB strains against 10 phytopathogens

from the genera *Pectobacterium*, *Streptomyces*, *Fusarium*, *Alternaria*, *Phoma*, *Rhizoctonia*, *Colletotrichum*. Different strains of *L. plantarum* were also reported as being strongly active towards *Rhodotorula mucilaginosa* and *P. brevicompactum* [59], *Botrytis cinerea* [60], *A. fumigatus* and *Rhizopus stolonifer* [33], *A. flavus*, *A. niger, Mucor circinelloides*, and *F. verticillioides* [61]. In addition to *L. plantarum*, the literature data indicate the antifungal potential of other species, similar to this work. The strains selected for the study included isolates from the species *L. paracasei* and *L. buchneri*, which demonstrated antifungal activity towards fungi belonging to *Fusarium*, *Alternaria*, *Sclerotinia*, *Colletotrichum,* and *Rhizoctonia* genera. Similarly, in the research of Kharazian et al. [62], different lactobacilli, including *L. buchneri* isolated from corn silages, showed antifungal activity towards *F. verticillioides*, *Penicillium* sp., and *Verticillium dahlia*. Lačanin et al. [59] selected the strain *L. paracasei* SYR90 together with two others, *L. plantarum* OVI9 and *L. rhamnosus* BIOIII28, as one of the three most promising strains. The antifungal potential of *L. paracasei* strains was also confirmed by Barrios-Roblero et al. [63], who showed antifungal activity of different LAB strains against *Colletotrichum gloeosporioides*, as well as Ramos-Pereira [64], who screened LAB for their activity against *Penicillium nordicum*, *P. commune,* and *P. verrucosum*.

Despite numerous in vitro studies demonstrating the antifungal properties of bacteria, as well as many works describing the possibilities of their use in food biopreservation, there are still limited data describing the use of LAB as biocontrol agents in plant protection in vivo. In the presented paper, five strains of LAB were tested for their effectiveness in the control of some fungal diseases of rye and wheat, both in mini-plot and field conditions. The observations concerned the common bunt of wheat and stalk smut rye in the mini-plot experiment, while the field experiments additionally included the effect of LAB on the seedling blight of rye and wheat as well as on emergence, overwintering, and yield grain. The results showed high efficacy of LAB in the control of common bunt of wheat and stem smut rye, with better results observed in the case of rye disease both in mini-plot and field conditions. Much lower effectiveness was observed in limiting the development of the seedling blight. The efficacy of the tested strains was different; however, it is quite difficult to select one of them as the best strain, although *L. plantarum* A7 seems to be the best candidate as a biological control agent.

The literature data confirm the potential of LAB as biological control agents. Byrne et al. [65] evaluated the effect of six LAB isolates on the development of *Fusarium* head blight under in vitro and greenhouse conditions. In a greenhouse, LAB were used in the form of a spray applied on to barley spikelets prior to *Fusarium* spore application. The results showed a significant reduction in disease severity by five LAB isolates. A significant decrease in deoxynivalenol content in spikelets by *L. amylovorus* DSM20552 was also observed. López-Seijas et al. [66] chose the strains *L. plantarum* LPLUV10 and *L. paracasei* LPAUV12, isolated from wine, to evaluate their potential as biocontrol agents towards *F. oxysporum* in *Lycopersicon esculentum* plants. The authors applied LAV, adding it to the irrigation water, while *F. oxysporum* f. sp. *lycopersici* CECT 2715 was added to the base of the plant. The results showed a significant reduction in the damaging effect of the fungus after the application of LAB compared to the control trial. Shrestha et al. [67] reported the efficacy of LAB against the bacterial spot pathogen (*Xanthomonas campestris* pv. *Vesicatoria*) in pepper (*Capsicum annuum* L. var. *annuum*) under greenhouse and field conditions. Their results showed a significant reduction in disease severity compared to untreated plants in both experiments. In turn, Mold Taha et al. [68] selected LAB strains belonging to *Weiseilla cibarra* and *Lactococcus lactis* subs. *Lactis* out of 230 endophytic bacterial isolates from papaya seed samples and tested their efficacy towards *Erwinia mallotivora*, responsible for papaya dieback disease. The authors inoculated papaya plants with the pathogen and selected LAB strains, using them as single strains and in the consortium. They observed the development of the disease for 30 days. The treatment reduced disease severity; however, the results were different. Disease suppression was higher with the bacterial consortium *W. cibaria* PPKSD19 and *L. lactis* subsp. *lactis* PPSSD39 than with strains PPKSD19 or PPSSD39 used alone. Steglińska et al. [58] described interesting results obtained after the application

of the selected strain KB2 LAB 03 on seed potatoes against some phytopathogens. The treatment caused a 40–90% reduction in infestation by eight pathogens, while *F. sambucinum* and *F. oxysporum* were not inhibited. However, it should be pointed out that the mentioned studies were carried out on potato samples in situ, not in under field conditions.

In the presented work, the treatment of wheat and rye seeds with LAB could increase grain yield and, in some cases, the weight of 1000 seeds. It is worth noting that the effect was strain-dependent; therefore, it was impossible to select the best strain-enhancing plant yield. It is important that phytotoxicity was not observed, which indicates that there is no potentially harmful effect of bacteria on the plants. Similar observations have been made by other authors, who reported some plant growth-promoting properties of LAB. Hamed et al. [69] tested the efficacy of LAB isolated from milk and yogurt against some phytopathogenic fungi invading tomato plants under in vitro and in vivo tests. The authors applied a culture broth of bacteria as the seed treatment or soil drench for *Fusarium oxysporum* and observed a plant growth-promoting effect apart from the antifungal activity of LAB. Similarly, López-Seijas et al. [66] stated that the *L. plantarum* LPLUVI10 and *L. paracasei* LPAUVI12 strains could promote the growth of tomato plants. The authors observed a significant increase in the dry weight of plants inoculated with *L. plantarum* LPLUV10 compared to untreated trials. Similarly, in the study of Zebboudj et al. [70], three LAB strains (*Lactobacillus delbruckii* subsp. *Bulgaricus*, *Leuconostoc mesenteroides* subsp. *Dextranicum*, and *Lactococcus lactis* subsp. *diacetylactis*) were tested for their antifungal activity towards *Fusarium* responsible for tomato crown and root rot under in vitro and in vivo conditions. The results confirmed the capacity of the examined LAB to inhibit phytopathogenic fungi growth, with *L. mesenteroides* significantly more efficient than other strains. Moreover, the authors observed a positive effect of LAB on the development of plant roots. Shrestha et al. [67] observed a plant growth-promoting effect of LAB on pepper when they used them as biological control agents; however, the results were varied and depended on the bacterial strain. Some strains had a significant effect on growth promotion, while others did not influence plants. It is noteworthy that some authors use the term "plant probiotics" for microorganisms enhancing growth and suppressing plant diseases [71].

5. Conclusions

Biological control and the search for new biocontrol agents are gaining more and more interest. Based on the presented work, we can conclude that LAB may be promising candidates as biological control agents; however, their efficacy is differentiated and depends on the strain and examined disease. Comparing the obtained results, the *L. plantarum* A7 strain demonstrated the best biocontrol potential among the tested LAB.

To the best of our knowledge, this is the first report showing the possibility of using LAB as biological agents towards some devastating plant diseases of wheat and rye. This is also one of only a few research projects describing field experiments with LAB. There is a clear need to expand research in this area because it is significantly limited and concerns selected diseases and selected experimental conditions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture14010061/s1, Table S1. Indicator microorganisms used for the antifungal activity studies; Table S2. Cultivation conditions and soil characteristics in the field experiments; Table S3. General information about fertilization and plant control; Table S4. Meteorological data during the field experiment in the sowing season 2015/2016; Table S5. Meteorological data during the field experiment in the sowing season 2016/2017; Table S6. Identification of selected strains based on proteomic and genetic profiles; Table S7. 16S rRNA gene sequences for selected LAB isolates; Table S8. The effect of LAB treatment on phytotoxicity (%). Figure S1. Fungistatic activity of tested LAB isolates against *F. graminearum* (C—control; P7.Z, P7.L, P7, A2.P, P40, P41, P42, G13, S12, A7—LAB isolates); Figure S2. Fungistatic activity of tested LAB isolates against *F. culmorum* (C—control; P7.Z, P7.L, P7, A2.P, P40, P41, P42, G13, S12, A7—LAB isolates); Figure S3. Fungistatic activity of tested LAB isolates against *F. poae* (C—control; P7.Z, P7.L, P7, A2.P, P40, P41, P42, G13, S12, A7—LAB isolates); Figure S4. Fungistatic activity of tested LAB isolates against *F. oxysporum*

(C—control; P7.Z, P7.L, P7, A2.P, P40, P41, P42, G13, S12, A7—LAB isolates); Figure S5. Fungistatic activity of tested LAB isolates against *R. solani* (C—control; P7.Z, P7.L, P7, A2.P, P40, P41, P42, G13, S12, A7—LAB isolates); Figure S6. Fungistatic activity of tested LAB isolates against *A. alternata* (C—control; P7.Z, P7.L, P7, A2.P, P40, P41, P42, G13, S12, A7—LAB isolates); Figure S7. Fungistatic activity of tested LAB isolates against *C. gleosporioides* (C—control; P7.Z, P7.L, P7, A2.P, P40, P41, P42, G13, S12, A7—LAB isolates); Figure S8. Fungistatic activity of tested LAB isolates against *S. sclerotiorum* (C—control; P7.Z, P7.L, P7, A2.P, P40, P41, P42, G13, S12, A7—LAB isolates).

Author Contributions: Conceptualization, R.G., D.G., K.K. and K.J.; methodology, D.G., R.G., K.J. and K.M.; software, K.J.; validation, K.J. and K.K.; formal analysis, R.G., K.K., D.G., K.J. and K.M.; investigation, R.G., K.K., D.G., K.J. and K.M.; resources, R.G. and D.G.; data curation, D.G., K.J. and K.M.; writing—original draft preparation, R.G., D.G., K.J. and K.M.; writing—review and editing, R.G., D.G., K.J. and K.M.; visualization, R.G., D.G., K.J. and K.M.; supervision, D.G. and R.G.; project administration, D.G. and K.J.; funding acquisition, R.G. and D.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in the article.

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

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