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RESEARCH ARTICLE

Biocontrol of Winter Wheat Root Rot by Rhizosphere Microorganisms in Kazakhstan

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Abstract: Root rot remains one of the most significant diseases of winter wheat (*Triticum aestivum* L.) in Kazakhstan, resulting in substantial yield losses. This study aimed to isolate and identify rhizosphere microorganisms associated with winter wheat and evaluate their antagonistic activity against root rot pathogens in the Almaty region of Kazakhstan. In total, seven bacterial and fungal isolates, belonging to the genera *Bacillus*, Lysinibacillus, and Trichoderma, were identified. Strain K-C-24 showed 99.87% homology with *Bacillus mojavensis*. *Trichoderma asperellum* strains GL and 101 showed the highest antagonistic activity, effectively inhibiting the growth of all tested pathogens, with inhibition reaching zones up to 37 mm. Bacterial strain K-C-24 (*B. mojavensis*) was active against *Bipolaris sorokiniana* with an inhibition zone of 23.5±0.5 mm, and its culture filtrate showed a strong inhibitory effect on *F. oxysporum* (42±0.5 mm), but showed no activity against other pathogens. Other isolates showed no significant antagonistic activity. The greenhouse experiment demonstrated the lowest rates of damage to the roots of wheat seedlings infected by *F. oxysporum* when treated with a consortium of bacteria from the genera *Bacillus* and *Lysinibacillus* (Mix bac) and the strain *T. asperellum* GL. The obtained results confirm the potential of using local antagonist strains, especially *T. asperellum, B. mojavensis,* and a consortium of bacteria, *Bacillus* and *Lysinibacillus* (Mix bac) strains, for the development of environmentally safe biopreparations against root rot of winter wheat in the Almaty region of Kazakhstan.

Keywords: Winter Wheat, Root Rot, Biological Control, Rhizosphere Microorganisms, Antagonistic Activity, T. Asperellum

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Introduction

Root rots caused by a complex of soil-borne phytopathogens pose a serious threat to global wheat production. According to recent studies [1], these diseases cause annual yield losses of 15-30%, resulting in significant economic losses. In Kazakhstan, where wheat is a strategically important crop, the problem is exacerbated by the prevalence of monoculture farming and changing climatic conditions [2].

The traditional plant protection system based on the use of chemical pesticides [3] is becoming less efficient due to the development of pathogen resistance and the accumulation of toxic residues in agroecosystems [4]. Alternative approaches, such as developing resistant varieties [5], have limited application due to the insufficient number of locally adapted varieties.

In this context, biological control using antagonistic microorganisms represents a promising approach [6]. Of particular interest are fungi of the genus Trichoderma [7], which exhibit direct antagonism through mycoparasitism and the synthesis of antimicrobial compounds [8], along with rhizobacteria (Bacillus, Pseudomonas), which possess multiple mechanisms of antagonism [9] and the ability to colonize the plant rhizosphere in soil. The effectiveness of biopreparations in field conditions has also been documented [10].

In Kazakhstan, where Fusarium spp. and Bipolaris sorokiniana (Sacc.) Shoemakers are the dominant pathogens [11, 12], and the development of biological control measures requires special attention. However, existing studies on autochthonous antagonist strains remain limited [13].

This study aims to identify the diversity of rhizospheric microbial antagonists associated with winter wheat and to evaluate their antagonistic potential against root rot pathogens occurring in the southeast of Kazakhstan. The scientific novelty lies in conducting the first comprehensive study assessing the biocontrol potential of the winter wheat rhizosphere in this region of Kazakhstan, identifying local strains with pronounced antagonistic activity, and establishing the scientific foundation for developing biopreparations. The practical significance includes the potential to reduce pesticide use, increase agroecosystem sustainability, and advance domestic biotechnology development. Our hypothesis proposes that the rhizosphere of winter wheat in the Almaty region harbors autochthonous microbial strains with high biocontrol potential, suitable for creating effective, locally adapted biopreparations.

Materials and Methods

Collection of Material and Isolation of Microorganisms

Field studies were conducted in August 2024 on winter wheat crops (varieties Bogarnaya 56, Steklovidnaya 24, and hybrids 231, APP) at the farm "Manshuk" (Almaty region, Kazakhstan; coordinates: 43.449794 N, 77.568206 E). Rhizosphere soil and plant roots were sampled during the full ripeness stage. Samples were transported in sterile paper bags at -20 °C. Sampling was conducted once in August 2024 at the full ripeness growth stage (BBCH 89), when rhizosphere microbial communities are considered relatively stabilized. Although seasonal variation in microbial composition is recognized as a significant factor, this study focused on late-season communities associated with mature plants. Future multi-season sampling will be required to assess temporal variability.

Isolation of bacteria, as in the case of fungi, was carried out by traditional methods with minor modifications [14]: 1 g of soil was suspended in 100 ml of sterile physiological solution (0.85% NaCl) and homogenized. Serial tenfold dilutions were prepared up to 10^6. Aliquots (100 µl) of the 10^{-4} to 10^{-6} dilutions were plated onto Meat-Peptone Agar (MPA) and Nutrient Agar (NA). Plates were incubated at 28±1°C for 24–48 h. Three to five colonies of each distinct morphotype were selected and purified.

Fungal isolation was performed as follows: Winter wheat root parts displaying root rot symptoms were surface-sterilized sequentially in 70% ethanol (1 min) and 3% NaOCl (3 min), followed by three rinses with sterile water. Sterilized root segments were placed on Potato Dextrose Agar (PDA) supplemented with chloramphenicol (50 mg/L). Plates were incubated at 25±1 °C for 5–7 days. Fungal isolates were then purified by the hyphal tip transfer method.

Additionally, five strains of Trichoderma asperellum Samuels, (1K, AHT, 11, 101, GL) and Fusarium oxysporum Schltdl. 17T from the collection of the Scientific Production Center of Microbiology and Virology were included.

Identification of Microbial Samples

Microorganisms were preliminarily identified based on morphological and culture characteristics by comparison with microbial atlases [15].

For molecular identification of bacteria, the universal 16S rRNA gene primers (Forward 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3′ and Reverse 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3′) were used. PCR amplification was

performed in a 25 µL reaction volume containing: 12.5 µL of 2× PCR master mix, 5 µL of bacterial DNA template, 1 µL of each primer (10 µM), and 5.5 µL of nuclease-free water. Amplification conditions were: initial denaturation at 94°C for 5 min; followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 100 s; with a final extension at 72°C for 10 min [16-18].

The PCR products were sequenced, and homology analysis was performed using the BLAST tool (NCBI). A phylogenetic tree was constructed using the neighbor-joining method in MEGA 11 [19].

Fungi were identified similarly. Approximately 1–2 mm² of mycelial mass was suspended in lysis buffer, incubated at 37 °C for 45 min, and then heated to 95 °C for 10 min. After centrifugation (13,000 × *g* for 2 min), the supernatant was used directly for PCR or stored at -70°C. Primer sequences (Forward and Reverse primers) and PCR conditions were used according to the manufacturers' protocols [20, 21]. The ITS region was amplified using universal primers ITS1 (5′ -TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′ -TCCTCCGCTTATTGATATGC-3′). PCR reactions (25 µL) contained 12.5 µL 2× master mix, 1 µL of each primer (10 µM), 2 µL DNA template, and nuclease-free water. Thermal cycling conditions were: 95 °C for 5 min; 35 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min; final extension at 72 °C for 7 min. Amplified products were visualized on a 2% agarose gel stained with ethidium bromide. PCR amplicons were purified, sequenced using the ABI BigDye Terminator v3.1 Cycle Sequencing Kit on an Applied Biosystems capillary sequencer, and analyzed using the Basic Local Alignment Search Tool (BLAST) against the NCBI nucleotide database (http://www.ncbi.nlm.nih.gov/BLAST/).

Determination of the Inhibitory Activity of Bacteria

The antagonistic activity of bacterial strains was evaluated using the dual-culture method [22]. Initially, a pathogen culture was spread uniformly over the entire surface of a Petri dish containing Potato Dextrose Agar (PDA) using a sterile spatula. Then, a well (8 mm in diameter) was created in the center of the plate using a sterile cork borer. One hundred microliters (100 µL) of the test bacterial culture was inoculated into this central well [23].

The experiment was carried out in three repetitions. The experiment was performed in triplicate. Plates were incubated at 27°C for 4 days. Antagonistic activity was assessed by measuring the diameter of the inhibition zones in two perpendicular directions and calculating the mean value.

Evaluation of Culture Filtrate Activity

The inhibitory activity of culture filtrates from selected microorganisms was assessed. Promising strains were cultured for 48 h at 37°C and 180 rpm in 100 mL of Meat-Peptone Broth (MPB) within 250 mL flasks. The broth was centrifuged at 10,000 × *g* and 4°C for 10 min. The supernatant was sterile-filtered through a 0.22 µm membrane filter and stored at 4°C [24, 25].

For the assay, the pathogen F. oxysporum Schltdl. 17T was first grown on PDA. Petri dishes containing PDA were uniformly inoculated with F. oxysporum using a sterile spreader. A well (approximately 6-8 mm diameter) was then created in the center of each plate using a sterile cork borer. An aliquot (e.g., 100 µL) of the sterile-filtered culture filtrate was pipetted into the central well. PDA plates inoculated with F. oxysporum 17T but without added filtrate served as negative controls. Plates were incubated for 2–3 days at 27°C. Following incubation, the diameter of the inhibition zone around the central well was measured in two perpendicular directions and the mean calculated. Alternatively, plates were photographed, and the area of pathogen growth inhibition was quantified using digital image analysis [22].

Pathogenicity Test

A pathogenicity test was performed to determine the virulence of the F. oxysporum 17T isolate (Scientific Production Center of Microbiology and Virology collection). A sterile substrate mixture of soil, sand, and vermiculite (1:1:1, v:v:v) was prepared. A conidial suspension of the pathogen, grown on PDA slants for 5 days, was adjusted to a titer of 10^7 conidia/mL (equivalent to approximately 250 conidia per gram of substrate). This suspension was thoroughly mixed into the substrate.

The inoculated substrate was transferred into sterilized plastic pots (9×12 cm). Pre-germinated seeds of spring wheat (Triticum aestivum L.) variety Steklovidnaya 24 were sown at a depth of 2 cm. Control pots received the same substrate mixture without pathogen inoculation. Pots were maintained in a growth chamber at 23°C with a 12-h photoperiod for 30 days.

Plants were watered regularly throughout the experiment. The test included three biological replicates per treatment (inoculated and control).

Pot Experiment

Plastic pots (9×12 cm) received 500 g of sterile substrate (soil: sand:vermiculite, 1:1:1 v/v; autoclaved at 121°C for 1 h, twice). Twenty-four hours before sowing, substrates were inoculated with 5 mL of F. oxysporum 17T conidial suspension (10^7 spores/mL). Surface-sterilized Triticum aestivum cv. 'Steklovidnaya 24' seeds (70% ethanol, 1 min; triple-rinsed with sterile H_2O) were bacterized/fungicized for 30 min with:

(i) Fungal antagonists: Trichoderma asperellum GL or T. simmonsii [26]

(ii) Bacterial treatments: Bacillus mojavensis or six-strain consortium "Mix bac" (B. mojavensis, B. mobilis, B. sanguinis, Lysinibacillus macroides [27], L. pakistanensis, L. fusiformis). The Mix bac consortium (Max bac) was formulated based on preliminary in vitro screening, where the six included strains demonstrated moderate antagonistic activity and complementary functional traits, including production of antibiotic compounds (Bacillus and Lysinibacillus spp), and strong sporulation capacity ensuring rhizosphere persistence. The combination was designed to enhance synergistic pathogen suppression under soil conditions. Twenty treated seeds per pot were sown (2 cm depth). Pathogen-exposed controls received sterile H_2O mock-inoculation. Employing a completely randomized design with five biological replicates, plants were grown at 23±1°C under a 14-h photoperiod, light intensity of approximately 250 µmol m^{-2} s^{-1}, and 70% relative humidity, with tri-weekly irrigation. After 30 days, root rot severity (visual assessment scale 0–5), root length, and shoot elongation were quantified. Root rot severity was evaluated using a 0–5 scale (0 = healthy roots; 1 = slight discoloration; 2 = moderate lesions <25%; 3 = 26–50% root necrosis; 4 = >50% necrosis; 5 = complete root destruction). Disease development index (DevI) was calculated using the formula

$$\mathrm{Dev}I\ (\%) = \frac{\sum_{i=1}^{k} sini}{SmaxN} \quad (1)$$

Si = Disease score in the i^{-th} category

Ni = Number of plants in the i^{-th} score category

K = Total number of score categories

Smax = Maximum disease score

N = Total number of assessed plants

Statistical Analysis

All data were subjected to one-way ANOVA followed by Tukey's HSD test (α = 0.0 5). Differences were considered significant at $p<0.05$. Values presented in tables represent mean ± standard deviation of three independent biological replicates.

Results

Collection of Material and Isolation of Bacteria and Fungi

Seven bacterial and five fungal isolates were obtained from soil and root samples of winter wheat varieties Bogarnaya 56, Steklovidnaya 24, and hybrid lines 231 and APP. Based on morphological and cultural characteristics (including colony color and morphology) and light microscopy analysis, bacterial isolates were assigned to the genera Bacillus and Lysinibacillus. Lysinibacillus cells were rod-shaped with characteristic ellipsoidal endospores. Gram staining confirmed that all isolates from both genera were Gram-positive. Bacillus isolates exhibited typical bacilliform morphology with cylindrical spores.

Fungal isolates were identified as members of the genera Fusarium, Alternaria, and Cladosporium based on distinctive colony coloration and morphology. In antagonistic activity assays, collection strains of T. asperellum (GL and 101) from the Scientific and Production Center of Microbiology and Virology demonstrated pronounced inhibitory activity against key phytopathogens: Alternaria sp., F. oxysporum 17T, Cladosporium sp., and *B. sorokiniana* (Figure 1).

The inhibition zone observed in Figure 1 suggested a combined mechanism of antagonism. Microscopic observations indicated hyphal deformation at the interaction zone, consistent with mycoparasitic activity. Additionally, the presence of a clear diffusion halo supports the involvement of extracellular metabolites contributing to antibiosis.

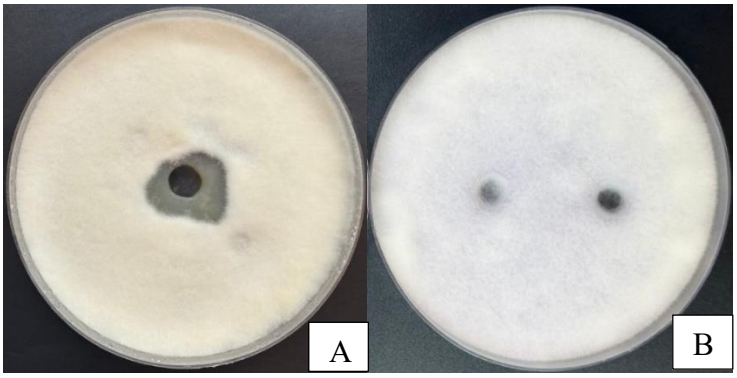

Fig. 1: Antagonistic activity of T. asperellum GL strain against F. oxysporum on diffusion agar plate (SPC of microbiology and virology, 2024). A - Inhibition zone formed by the fungal isolate; B - Control sample without fungal inoculation

Identification of Isolates

All bacterial isolates cultured on MPA exhibited consistent colony morphology, characterized by circular form with smooth margins, uniform milky-white coloration, and glossy surfaces without pigmentation. For precise taxonomic identification, we performed 16S rDNA gene amplification by PCR followed by sequencing, with subsequent analysis using the NCBI BLAST database.

Comparative analysis of the 16S rDNA sequences using the NCBI BLAST database identified the bacterial isolates as: B. mojavensis, B. mobilis, B. sanguinis, L. macroides, L. pakistanensis, and L. fusiformis. Boot analysis (1000 replicates) showed strong support (>95%) for clustering of strain K-C-24 with reference B. mojavensis strains (Figure 2), conforming its taxonomic placement and excluding closely related Bacillus species.

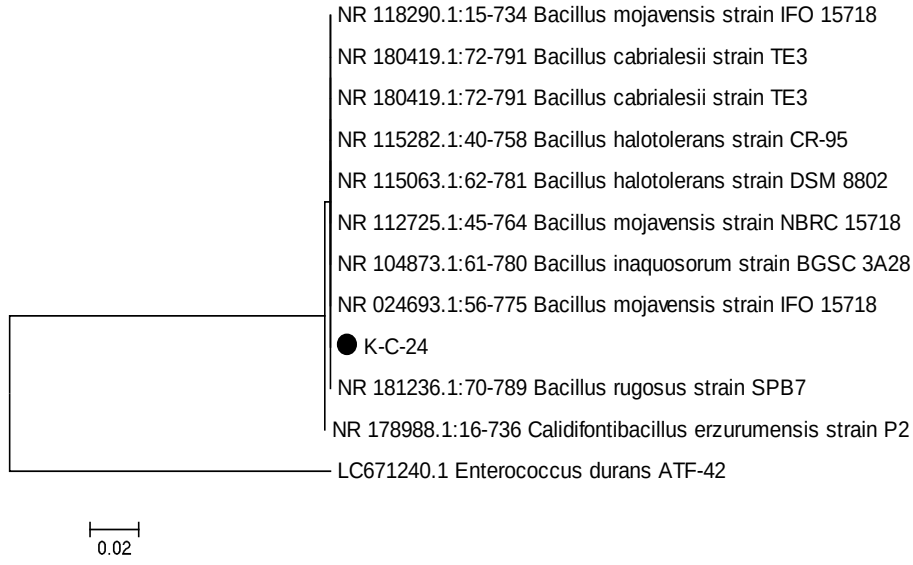

Fig. 2: Phylogenetic tree of strain K-C-24, identified as B. mojavensis strain IFO 15718, with a sequence similarity of 99.86% (SPC of microbiology and virology, 2024)

The close clustering of strain K-C-24 with representatives of B. mojavensis, together with its clear separation from other species of the genus Bacillus (Bacillus rugosus SPB7 and Bacillus inacuosorum BGSC 3A28), supports its phylogenetic coherence and confirms the accuracy of its taxonomic assignment.

Screening of Microorganism Activity

The efficiency of isolated microorganisms against phytopathogenic fungi was evaluated by the double culturing method. In the process of testing 7 strains of bacteria and fungi of the Trichoderma genus isolated from wheat rhizosphere, as well as 5 collection strains of fungi of the Trichoderma genus against test phytopathogens: Alternaria sp., F. oxysporum 17T, Cladosporium sp., and *B. sorokiniana*, it was found that among all bacterial strains, only spore flush K-C-24 B. mojavensis showed a slight antagonistic activity with a zone of action of 23 mm against the *B. sorokiniana* strain (Fig. 3).

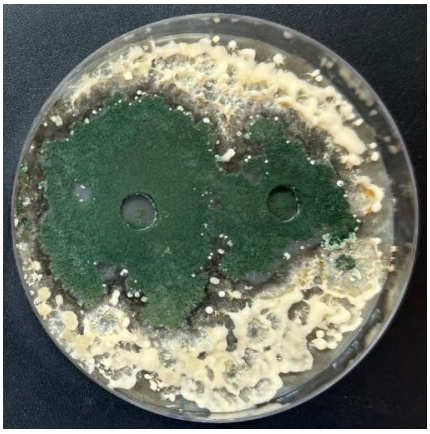

Fig. 3: Antagonism of T. asperellum GL strain against *B. sorokiniana* as test pathogen strain

Table 1 below demonstrates the lack of effect in the spore flush of the B. mojavensis strain against F. oxysporum 17T, Alternaria sp., and Cladosporium sp. Among the fungal tested strains, based on conidial flushes of the collection strains T. asperellum, the GL and 101 isolates showed significant inhibitory activity against all the tested pathogens, including F. oxysporum 17T, Alternaria sp., Cladosporium sp., and *B. sorokiniana*, with suppression zones up to 37 mm (Table 1). However, of these two strains, only T. asperellum GL had the highest zones of inhibition against all tested pathogens, and especially against Cladosporium sp and *B. sorokiniana*.

Table 1: Antagonistic activity of isolated bacterial and fungal isolates against wheat phytopathogens (in vitro) by size of suppression zones (SPC of Microbiology and Virology, 2024)

Antagonistic strains	Pathogens tested, Inhibition zone/mm			
	Fusarium sp	Alternaria sp	Cladosporium sp	*B. sorokiniana*
Bacteria				
B. mojavensis K-C-24	no growth	no growth	no growth	23.5± 0.5
L. fusiformis D-bac4	no growth	no growth	no growth	no growth
L. pakistanensis A-bac1	no growth	no growth	no growth	no growth
L. pakistanensis U-12-L	no growth	no growth	no growth	no growth
L. macroides F-bac alter2	no growth	no growth	no growth	no growth
B. mobilis E-bac alter1	no growth	no growth	no growth	no growth
B. sanguinis C-bac3	no growth	no growth	no growth	no growth
Fungi				
T. asperellum GL	24.5± 0.5	23± 0.5	36± 0.5	37± 0.5
T. asperellum101	13 ±0.5	24.3± 0.5	18.3± 0.5	26± 0.5

Evaluation of the Culture Broth Components' Activity

The culture broth of the tested 6 bacterial and 1 fungal strain, cultured for 5 days, showed a heterogeneous pattern of their inhibitory activity. Of all 6 bacterial isolates and the T. simmonsii strain, only the supernatant, i.e. supernatant, and the culture broth itself of the B. mojavensis K-C-24 strain showed a significant zone of inhibition of the test pathogen F. oxysporum 17 T (Figure 4).

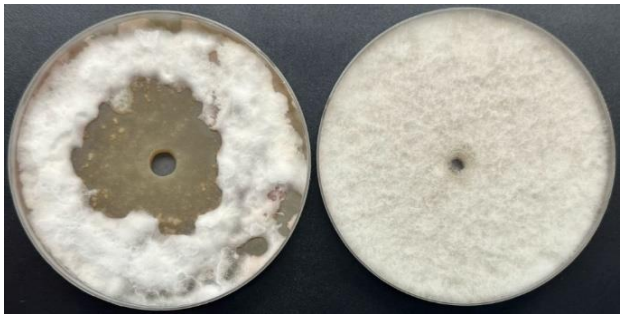

Fig. 4: Antagonism of the B. mojavensis K-C-24 strain against F. oxysporum

Table 2 shows that the culture broth supernatant of the K-C-24 (B. mojavensis) strain demonstrated significant antagonistic activity, inhibiting the growth of F. oxysporum 17T with an inhibition zone of 42±0.5 mm. Other bacterial strains, such as L. fusiformis, L. pakistanensis, L. macroides, B. mobilis, and B. sanguinis, as well as the fungus T. simmonsii, did not show inhibitory activity against the tested phytopathogens, which confirms their limited biocontrol potential. (Table 2). This fact of the pronounced activity of the supernatant of the B. mojavensis strain (Table 2) indicates that the suppression of F. oxysporum 17T is due to an antibiotic mechanism (antibiosis) associated with the secretion of diffusing metabolites. At the same time, these results indicate that the main mechanism of suppression of phytopathogens by fungi of the genus Trichoderma is associated not with soluble metabolites, but with mycoparasitism, contact destruction of pathogen hyphae, and competition for substrate and colonization advantage.

Table 2: Inhibitory activity of culture broth supernatant of antagonistic strains against wheat phytopathogens (in vitro), mm zone of suppression (SPC of Microbiology and Virology, 2024)

Antagonistic strains	Pathogens tested, Inhibition zone/mm			
	F. oxysporum	Alternaria sp	Cladosporium sp	*B. sorokiniana*
	Bacteria			
B. mojavensis K-C-24	42± 0.5	no growth	no growth	no growth
L. fusiformis D-bac4	no growth	no growth	no growth	no growth
L. pakistanensis A-bac1	no growth	no growth	no growth	no growth
L. macroides F-bac alter2	no growth	no growth	no growth	no growth
B. mobilis E-bac alter1	no growth	no growth	no growth	no growth
B. sanguinis C-bac3	no growth	no growth	no growth	no growth
	Fungi			
Trichoderma simmonsii	no growth	no growth	no growth	no growth

Notably, while B. mojavensis K-C-24 showed limited inhibition in dual culture (Table 1); its culture filtrate exhibited strong activity against F. oxysporum (Table 2), indicating that diffusible metabolites rather than direct competition are the primary antagonistic mechanism for this strain

Pathogenicity Test

The pathogenicity of F. oxysporum was evaluated on spring wheat seedlings under laboratory conditions. The pathogen was consistently isolated from symptomatic plants, purified in axenic culture, and used for artificial inoculation. Inoculated seedling developed typical root rot symptoms, and the same pathogen was successfully re-isolated from affected tissues, thereby fulfilling Koch's postulates.

Artificial inoculation with F. oxysporum 17T resulted in s statistically significant increase ($P<0.01$) in both disease incidence and severity compared with the non-inoculated control (Table 3). In cultivar I-1, inoculated seedlings showed a disease incidence of 40.0% and a disease severity index of 16.7%, whereas in the non-inoculated control, these values were 10.0% and 2.5%, respectively. In cultivar Diva, higher susceptibility was observed, with incidence and severity reaching 67.7% and 23.7% following inoculation, compared 15.0% and 3.0% in the control. In control variants, only a low background level of root rot was recorded. This result confirms the pathogenicity of F. oxysporum to spring wheat and demonstrates cultivar-dependent differences in susceptibility.

Table 3: The degree of infection of spring wheat seedlings by the pathogen *F. oxysporum* 17T sprouted in pots under laboratory conditions

Variant	Distribution index (ID, %)	Development index (ID, %)
Variety I-1 (inoculated with F. oxysporum)	40.0	16.7
Variety I-1 (not inoculated with F. oxysporum)	10,0	2.5
Variety Diva (inoculated with F. oxysporum)	67.7	23.7
Variety Diva (non-inoculated with F. oxysporum)	15,0	3,5
P value	<0.01	<0.01

Pot Assay

The results on the effect of biological preparations on the infestation of spring wheat seedlings with the F. oxysporum pathogen are presented in Table 4. The consortium of 6 bacterial species (Mix bac) showed the greatest effectiveness, which significantly ($p<0.01$) reduced both the development index (Dev_I - 46.51%) and the distribution index (Dis_I - 25.00%) of the disease compared with the control (Dev_I - 60.34%, Dis_I - 37.93%). The bioproduct based on T. asperellum GL also demonstrated a significant protective effect (Dev_I - 52.11%, Dis_I - 35.92%), while T. simmonsii (Dev_I - 62.30%, Dis_I - 41.80%) and B. mojavensis (Dev_I - 72.97%, Dis_I - 37.84%) proved to be less effective. Statistically significant differences between the variants ($p<0.01$) confirm the effect of bioproducts on the degree of damage to plants.

Table 4: Effect of biological products on the degree of infestation of spring wheat seedlings with F. oxysporum pathogen

Variant	Development Index (Dev_I, %)	Distribution index (Dis_I, %)
T. asperellum GL	52.11	35.92
T. simmonsii	62.30	41.80
B. mojavensis	72.97	37.84
Mix bac (consortium of 6 species)	46.51	25.00
Control	60.34	37.93
P value	<0.01	<0.01

Discussion

In this study, bacterial and fungal isolates isolated from the soil and rhizosphere of winter and spring wheat, as well as several strains of fungi from the collection, were analyzed in order to determine their antagonistic activity against the pathogens F. oxysporum and *B. sorokiniana*.

The ability of Bacillus strains not only to suppress phytopathogens [28, 29] but also to stimulate plant growth [30, 31], reduce the content of mycotoxins [32], and have an anti-insect effect [33] is well-known. In this in vitro study, the B. mojavensis K-C-24 strain was the only one among bacterial isolates of this genus that showed pronounced inhibitory activity against F. oxysporum. The data obtained are consistent with the results of previous studies, where strains of B. mojavensis have demonstrated similar efficacy [34-37]. Such antagonistic activity against the pathogen can manifest itself not only in the production of hydrolytic enzymes [38] but also, in general, contribute to an increase in plant growth, grain quality, soil nutrient dynamics, and microbial activity [39].

Members of the genus Lysinibacillus, formerly classified as Bacillus, are known for their ability to affect crops positively [40]. This similarity of the two groups of organisms is also revealed in the antiphytopathogenic activity. For example, the L. fusiformis S4C11 strain, known for its ability to produce VOCs, inhibits pathogens such as Aspergillus nigri Tiegh., Botrytis cinerea Pers., and Rhizoctonia solani J.G. Kühn [41]. Moreover, the activity of other strains of this species was expressed, for example, in improving the vegetative parameters of wheat [42], and phosphate solubilization and hormone production [43]. A similar effect against the above-mentioned pathogens is observed in strain L. pakistanensis [44]. Another mechanism of action is found in the L. macroides species, whose potential is revealed in the production of various enzymes that contribute to the destruction of the cell walls of pathogenic fungi [27, 45]. In turn, in the present study, the strains L. fusiformis, L.

pakistanensis, and L. macroides, which initially showed little antagonistic activity, did not show the expected activity in inhibiting test pathogens during screening.

Among the plant bioagents of the genus Trichoderma sp., T. asperellum strains are known for their high inhibitory effect. The bioregulatory effect of these microorganisms in vitro is capable of competing for food and a niche, destroying fungal cell walls using secreted enzymes, secondary metabolites, and causing Systemic Resistance (ISR) in plants [46-50]. In the present study, the T. asperellum GL and 101 strains show the highest rates of antagonistic activity among the tested strains, not only fungi, but also bacteria, when evaluated by screening and supernatant of culture filtrate, demonstrating the highest suppression zones of the tested pathogens, such as F. oxysporum 17T, *B. sorokiniana*, Alternaria sp., and Cladosporium sp.

Meanwhile, a potting experiment conducted in a greenhouse showed the predominance of bacterial strains Mix bac as a consortium over fungal strains (T. asperellum GL and T. simmonsii) in the antagonistic activity. Thus, the Mix bac strain had the lowest indices of development (Dev_I) and distribution (Dis_I) in comparison with the strains B. mojavensis, T. asperellum GL, and T. simmonsii. Additionally, a greenhouse experiment revealed the key moment of the action of antagonists in the soil of growing crops, namely their effectiveness on pathogenic fungi in joint activities. That is, despite the certain result achieved by the B. mojavensis strain in inhibiting, for example, the pathogen F. oxysporum performed in vitro, in comparison with other tested 5 bacterial species, their complex participation in the form of a consortium of mixed bacteria significantly increased the effect of antagonism of the fungus.

Thus, the results obtained for the present study confirm the effectiveness of the application of local antagonistic microorganisms, in particular a consortium of Bacillus and Lysinibacillus (Mix bac) bacteria species and T. asperellum GL strains, in the biological protection of grain crops against a complex of root rot pathogens.

Conclusion

1. Seven bacterial and five fungal isolates were isolated from the rhizosphere of winter wheat varieties Bogarnaya 56, Steklovidnaya 24, and hybrids 231 and APP. Morphological and molecular methods allowed us to identify bacteria as representatives of the genera Bacillus and Lysinibacillus, and fungi as representatives of the genera Fusarium, Alternaria, and Cladosporium
2. Sequencing of the 16S rDNA gene confirmed a high degree of homology of the bacterial isolates with known species; in particular, strain K-C-24 was assigned to B. mojavensis (99.87%)
3. Antagonistic activity testing showed that of the five Trichoderma spp strains, only T. asperellum GL and 101 strains showed the highest efficacy, being able to inhibit the growth of major phytopathogens of winter wheat, including F. oxysporum 17T, Alternaria sp., Cladosporium sp., and *B. sorokiniana*, with zones of inhibition up to 37 mm
4. The culture filtrate supernatant of strain K-C-24 (B. mojavensis) showed antagonistic activity against *B. sorokiniana*, with a zone of inhibition of 23.5 ± 0.5 mm, but showed no activity against other pathogens
5. A consortium of Bacillus and Lysinibacillus species (Mix bac) and T. asperellum GL strains significantly inhibited Fusarium infection of wheat seedlings in a greenhouse background

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Author's Contributions

Nurlan Kuldybayev: Conceptualization, drafting, and wrote the manuscript.
Amankeldy Sadanov: Funding acquisition and project administration.
Aigerim Qairatova: Collected field materials, daily isolation of microbial samples.
Zhanar Suleimenova: Contributed to daily investigation.
Gulnar Dzhakibayeva: Contributed to result interpretation.
Akmeiir Yelubayeva: Contributed to molecular identification.
Yerlan Dutbayev: Statistical analysis of the data, data interpretation, and final revision.

Ethics

This study involved plant material only and did not require approval from an animal ethics committee. All experimental procedures complied with institutional guidelines for plant research.

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