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Indigenous Putative Nitrogen-Fixing Bacteria from Northern Kazakhstan: Antagonistic Activity and Growth Promotion in Buckwheat

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Abstract

The present study aimed to evaluate the biocontrol and plant growth-promoting potential of putative nitrogen-fixing bacterial strains isolated from the soils of Northern Kazakhstan against major cereal crop phytopathogens and during the early stages of plant development. Phytopathogenic fungi, *Fusarium proliferatum* and *Alternaria alternata*, were isolated from infected cereal tissues and seeds using conventional mycological techniques. The antagonistic activity of the bacterial strains was assessed using a dual-culture assay. A total of 26 bacterial isolates were screened for antagonistic activity and plant growth-promoting effects. Several isolates exhibited pronounced inhibitory activity against both pathogens by suppressing fungal mycelial growth. The plant growth-promoting potential of bacterial culture filtrates was evaluated under in vitro conditions using buckwheat (*Fagopyrum esculentum* Moench) varieties 'Shortandinskaya 6' and 'Shortandinskaya Krupnozernaya'. Germination energy, laboratory germination, shoot length, and root length were recorded during seedling development. The tested bacterial culture filtrates showed a stimulatory effect on seedling growth, with the most pronounced response observed in root elongation, indicating their positive influence on rhizogenesis. The obtained results demonstrate that *Agrobacterium pusense* st.51S, *Ensifer fredii* st.59S, and *Oricola cellulosilytica* st.70S exhibited strong antagonistic activity against *Fusarium proliferatum*, whereas *O. cellulosilytica* st.70S, *Streptomyces virginiae* st.11S, and *Ensifer meliloti* st.56S effectively inhibited the growth of *Alternaria alternata*. In addition, *E. meliloti* st.56S, *Priestia megaterium* st.35S, *Rhizobium giardinii* st.2S, and *Ensifer sp.* st.91S significantly promoted buckwheat seed germination and seedling growth. The most pronounced growth-promoting effect was observed for *E. meliloti* st.56S, which increased shoot and root length of 'Shortandinskaya 6' by 31.4% and 23.8%, respectively, compared with the control. These strains represent promising candidates for the development of microbial bioformulations aimed at improving seed quality, enhancing early plant development, and promoting sustainable crop production under the dry steppe conditions of Northern Kazakhstan.

Keywords: plant growth-promoting bacteria; biocontrol; rhizobacteria; antagonistic activity; phytopathogenic fungi

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1. Introduction

Nitrogen is one of the essential macronutrients required for plant growth and development, as it is a fundamental component of amino acids, proteins, nucleic acids, and chlorophyll [1]. In modern agriculture, nitrogen deficiency is commonly compensated through the application of synthetic nitrogen fertilizers [2]. However, the excessive use of chemical fertilizers has resulted in numerous environmental concerns, including soil degradation, water pollution, greenhouse gas emissions, and increased production costs [3]. Consequently, the development of environmentally friendly and sustainable alternatives to mineral nitrogen fertilization has become a major objective of modern agriculture [4].

One of the most promising approaches is the use of plant-associated diazotrophic bacteria for biological nitrogen fixation. These microorganisms convert atmospheric nitrogen into forms available for plant uptake, thereby improving nitrogen nutrition while reducing dependence on synthetic fertilizers [5]. Nitrogen-fixing bacteria include free-living, associative, and endophytic microorganisms belonging to the genera *Azospirillum*, *Azotobacter*, *Beijerinckia*, and several other taxonomic groups [6]. Their beneficial role has been extensively investigated in symbiotic associations with legumes, where atmospheric nitrogen fixation occurs within root nodules [7]. In contrast, non-leguminous crops, including buckwheat, rice, and maize, do not establish classical nitrogen-fixing nodules. Instead, they rely on associative and free-living diazotrophic bacteria inhabiting the rhizosphere or internal plant tissues [8].

Recent studies have demonstrated that plant-associated nitrogen-fixing bacteria provide multiple benefits beyond biological nitrogen fixation. These microorganisms improve nutrient availability, synthesize phytohormones, enhance nutrient uptake, stimulate plant growth, and increase plant tolerance to both abiotic and biotic stresses [9]. Many endophytic and rhizosphere bacteria produce indole-3-acetic acid (IAA), gibberellins, cytokinins, siderophores, and other biologically active metabolites that regulate plant development and promote seed germination [10,11]. Seed inoculation with plant growth-promoting diazotrophic bacteria, particularly *Azospirillum brasilense*, has been reported to accelerate seed germination and improve early seedling vigor through enhanced nitrogen availability and the production of biologically active microbial metabolites [12,13]. These effects are especially evident under controlled in vitro conditions, where environmental variability is minimized [14].

In addition to promoting plant growth, nitrogen-fixing bacteria may also function as biological control agents against phytopathogenic microorganisms [15]. Their antagonistic activity involves several complementary mechanisms, including competition for nutrients and ecological niches, production of antifungal and antibacterial metabolites, secretion of hydrolytic enzymes, synthesis of siderophores, and induction of systemic resistance in host plants [16]. These properties make diazotrophic bacteria promising candidates for the development of environmentally friendly microbial biocontrol products capable of reducing disease incidence while decreasing reliance on chemical fungicides [17].

Despite considerable progress in the application of plant growth-promoting bacteria, their effectiveness under different agricultural conditions remains inconsistent [18]. The efficiency of bacterial inoculants largely depends on the microbial strain, host plant genotype, environmental conditions, and interactions with indigenous soil microbial communities [19]. Although numerous studies have investigated the plant growth-promoting properties of diazotrophic bacteria, considerably less attention has been paid to the simultaneous evaluation of their antagonistic activity against cereal phytopathogens and their effects on early seed germination. Furthermore, information on indigenous putative nitrogen-fixing bacterial strains isolated from the dry steppe soils of Northern Kazakhstan remains scarce. Therefore, the identification and characterization of multi-

functional bacterial strains that combine biocontrol activity with plant growth-promoting properties remain an important research priority for sustainable crop production [20].

The present study aimed to evaluate the antagonistic activity of putative nitrogen-fixing bacterial strains isolated from the soils of Northern Kazakhstan against the phytopathogenic fungi *Fusarium proliferatum* and *Alternaria alternata*, as well as to assess the effects of their culture filtrates on seed germination and early seedling development of buckwheat (*Fagopyrum esculentum* Moench).

2. Materials and Methods

2.1. Study Area and Plant Material

Putative nitrogen-fixing bacteria were isolated from 15 composite soil samples collected from five representative sites in the dry steppe zone of Northern Kazakhstan during the growing season (May–June 2025). Soil samples were collected from the 0–20 cm layer using the envelope sampling method. At each site, five subsamples were randomly collected and pooled to form each composite sample, with three independent composite samples collected per site. The sampling sites were located at 51.7000° N, 71.0500° E (southern chernozem), 53.0823° N, 70.3069° E (ordinary chernozem), 53.1685° N, 69.1270° E (chernozem), and two additional sites representing transitional steppe soil types (Figure 1). The study area is situated at an altitude of 300–420 m above sea level and is characterized by a sharply continental climate with cold winters (minimum temperatures down to −30 °C), warm summers (maximum temperatures up to +30 °C), and low annual precipitation ranging from 250 to 350 mm. Water availability is the primary factor limiting crop productivity in the region.

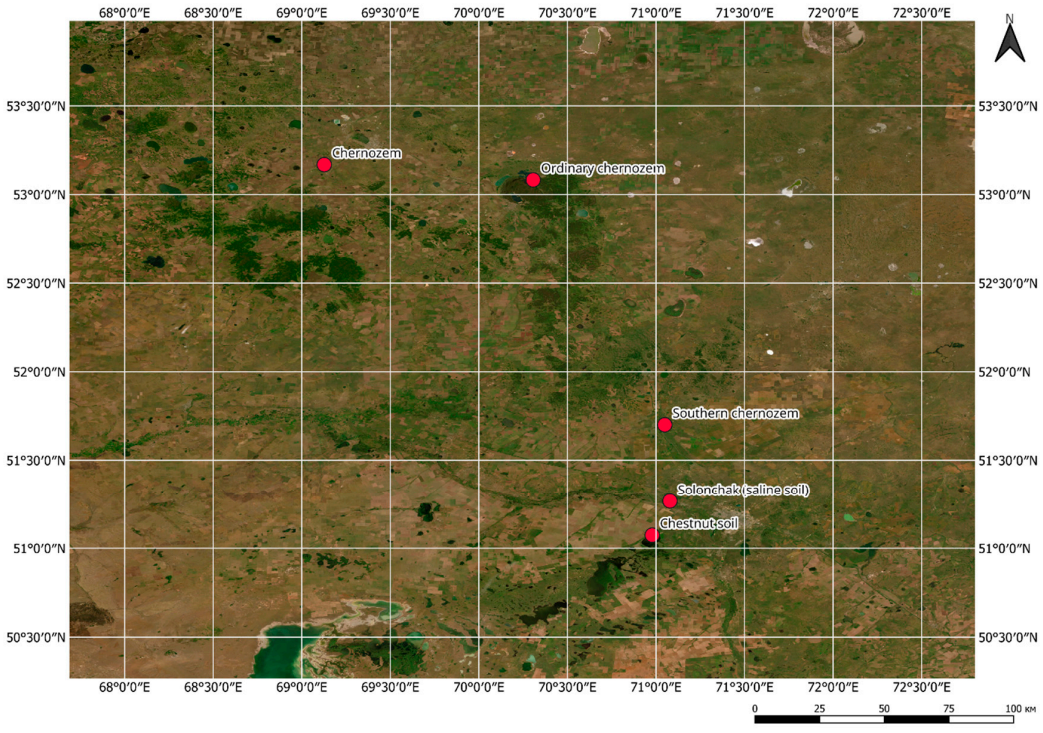

Figure 1. Study area and sampling points.

The dominant soil types include ordinary and southern chernozems and dark chestnut soils, which are typical of cereal-growing agroecosystems in Northern Kazakhstan. These soils are characterized by moderate humus content, variable nitrogen availability, and periodic moisture deficiency. The selected sampling sites are representative of the major rainfed cereal production region of Northern Kazakhstan. Therefore, the isolated

indigenous putative nitrogen-fixing bacteria represent the native soil microbiota naturally adapted to the soil and climatic conditions of the dry steppe.

Buckwheat (*Fagopyrum esculentum* Moench) varieties 'Shortandinskaya 6' and 'Shortandinskaya Krupnozernaya' were used as plant material. Seeds of both varieties were obtained from the A.I. Baraev Scientific and Production Center of Grain Farming, Kazakhstan. Both varieties are established buckwheat varieties and are not hybrids. They are widely cultivated in Northern Kazakhstan due to their adaptation to the soil and climatic conditions of the dry steppe zone.

The variety 'Shortandinskaya 6' is a medium-maturing variety characterized by high yield potential, resistance to lodging and seed shattering, and large, uniform seeds with good technological quality. The growing season lasts 95–98 days. Owing to its high seed viability and germination capacity, this variety is suitable for evaluating the effects of microbial inoculants on seed germination, early seedling growth, and plant productivity [21].

The variety 'Shortandinskaya Krupnozernaya' is also a medium-maturing variety characterized by high productivity, drought tolerance, and large grain size. The growing season lasts 80–83 days, and the thousand-kernel weight ranges from 30 to 35 g. Its well-filled and uniform seeds make this variety suitable for evaluating the effects of microbial inoculants on seed germination and early plant development [22].

Before the experiments, seeds were visually inspected, and mechanically damaged seeds were discarded. Only seeds with uniform size and weight were selected to minimize variation associated with seed quality.

2.2. Isolation and Identification of Microorganisms

Phytopathogenic fungi were isolated from infected vegetative tissues and seeds of cereal crops using standard mycological isolation techniques on potato-based sucrose agar medium containing 250 g of potato, 30 g of sucrose, and 16 g of agar per liter of distilled water [23]. Morphological characteristics of the fungal isolates, including mycelial structures, conidia, and conidiophores, were examined by light microscopy. Fresh fungal material was mounted in a drop of sterile distilled water on glass slides, covered with coverslips, and examined and documented under a light microscope [24].

Bacterial strains were isolated from soil samples using the serial dilution plate method. Briefly, 10 g of soil was suspended in 90 mL of sterile distilled water and thoroughly mixed. Serial ten-fold dilutions were prepared, and aliquots were plated onto nitrogen-free Ashby's medium, Gause's medium, and meat-peptone agar (MPA). Ashby's medium was prepared per liter of distilled water using 20 g of mannitol, 0.2 g of K_2HPO_4, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.2 g of NaCl, 0.1 g of K_2SO_4, 5 g of $CaCO_3$, and 16 g of agar. Gause's medium was prepared per liter of distilled water using 20 g of starch, 1 g of KNO_3, 0.5 g of K_2HPO_4, 0.5 g of $MgSO_4$, 0.5 g of NaCl, 0.01 g of $FeSO_4$, and 16 g of agar. Meat-peptone agar (MPA) was prepared by dissolving 28 g of the commercially available dehydrated medium (HiMedia, TM 1040) in 1 L of distilled water, followed by the addition of 16 g of agar. The medium was then sterilized by autoclaving according to the manufacturer's instructions. Morphologically distinct bacterial colonies were selected, repeatedly subcultured, and purified to obtain pure cultures. A total of 30 bacterial isolates were obtained, of which 26 isolates were subsequently selected for the antagonistic and plant growth-promoting assays. All 26 selected isolates were capable of growth on nitrogen-free Ashby's medium, which was used as a screening criterion for putative diazotrophic characteristics. Direct nitrogen fixation was not confirmed by an acetylene reduction assay or *nifH* gene analysis.

For molecular identification of the bacterial isolates, genomic DNA was extracted using the GeneJET PCR Purification Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol. A partial 16S rRNA gene region of approx-

imately 890 bp was amplified using primers 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 806R (5′-GGACTACCAGGGTATCTAAT-3′) [25]. PCR was performed in a final volume of 25 µL containing 25 ng of DNA template, 1 U of DNA polymerase, 0.2 mM of each dNTP, 1× PCR buffer, 2.5 mM of $MgCl_2$, and 10 pmol of each primer. Amplification was performed using a SimpliAmp thermal cycler (Thermo Fisher Scientific, Waltham, MA, USA), and PCR products were checked by electrophoresis on a 1.5% agarose gel in 1× TAE buffer. The amplified fragments were sequenced by the Sanger method using BigDye Terminator chemistry, with sequencing products analyzed on an ABI 3130XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Both forward and reverse primers were used for sequencing. Chromatograms were processed using Sequencing Analysis v5.2, and the resulting sequences were compared with sequences in the NCBI database using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 15 September 2026).

For molecular identification, genomic DNA was extracted from fungal cultures using a CTAB-based protocol. Approximately 0.1–0.2 g of fungal biomass was processed for DNA extraction, and the obtained DNA was quantified using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). The internal transcribed spacer (ITS) region was amplified using the universal fungal primers ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) [26]. PCR was performed in a final volume of 30 µL containing 5 µL of template DNA, 1 U of Taq DNA polymerase, 0.2 mM of each dNTP, 10× KCl buffer, 2.5 mM of $MgCl_2$, and 10 pmol of each primer. The amplification program consisted of an initial denaturation at 94 °C for 5 min, followed by 30 cycles of 95 °C for 30 s, 55 °C for 40 s, and 72 °C for 50 s, with a final extension at 72 °C for 7 min. PCR products were verified by electrophoresis on a 1.5% agarose gel and purified using a magnetic silica-based method before sequencing. Sanger sequencing was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit on a 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Sequences were assembled and edited using SeqMan (DNASTAR; https://www.dnastar.com, accessed on 15 September 2026) and compared with sequences in the GenBank database using BLAST [27]. For phylogenetic analysis, the obtained ITS sequences were aligned with reference sequences using MUSCLE, phylogenetic trees were constructed in MEGA (https://www.megasoftware.net, accessed on 15 September 2026) X using the Neighbor-Joining (NJ) method, and node support was evaluated by bootstrap analysis with 1000 replicates.

The same ITS-based molecular workflow was applied to the identification of both *Fusarium* and *Alternaria* isolates. Because ITS sequence analysis may have limited resolution for distinguishing closely related species of *Fusarium* and *Alternaria*, the molecular identifications in this study were interpreted together with morphological characteristics, BLAST sequence similarity, and ITS-based phylogenetic analysis. The species assignments should therefore be considered ITS-based identifications rather than definitive multilocus taxonomic assignments.

2.3. Evaluation of Antagonistic Activity

The antagonistic activity of putative nitrogen-fixing bacterial strains against phytopathogenic fungi was evaluated using the dual-culture assay on the potato-based sucrose agar medium described above [28]. Pure cultures of fungal pathogens and bacterial isolates were cultivated separately on this medium at 25 °C until active growth was observed [29]. A fungal mycelial plug was placed on one side of a Petri dish, whereas the bacterial isolate was inoculated on the opposite side [30]. Plates were incubated at 25 °C for 5–7 days. Fungal growth toward the bacterial colony was measured and compared with the untreated control [31]. Antagonistic activity was expressed as either the width of the inhibition zone (mm) or the percentage of mycelial growth inhibition [32].

2.4. In Vitro Seed Germination Assay

The effects of cell-free bacterial culture filtrates on buckwheat seed germination were evaluated under in vitro conditions [33]. All 26 putative nitrogen-fixing bacterial strains were capable of growth in nitrogen-free Ashby's medium and were cultivated at 28 °C for 48–72 h with continuous shaking at 120 rpm. Growth on nitrogen-free medium was used as a screening criterion for putative diazotrophic characteristics. The cultures were centrifuged, and the supernatants were sterilized by filtration through 0.22 μm membrane filters to obtain cell-free culture filtrates containing extracellular microbial metabolites [34].

Buckwheat seeds were surface-sterilized with 0.1% potassium permanganate solution for 2 min and subsequently rinsed thoroughly three times with sterile distilled water. Seeds were placed on sterile filter paper in Petri dishes and treated either with bacterial culture filtrates or sterile distilled water (control). Germination was assessed by counting the number of germinated seeds on the 3rd and 7th days after the start of the experiment. Germination energy (GE, %) was calculated on day 3 as the percentage of seeds that had germinated relative to the total number of seeds tested: GE (%) = (number of germinated seeds on day 3/total number of seeds tested) × 100. Final germination percentage was determined on day 7 using the same calculation: germination (%) = (number of germinated seeds on day 7/total number of seeds tested) × 100 [35]. Shoot and root lengths were also measured.

2.5. Statistical Analysis

All experiments were performed in triplicate (n = 3). Data are presented as mean ± standard deviation (SD). Statistical analyses were performed using Minitab® 17 (Minitab LLC, State College, PA, USA). Differences among treatments were evaluated by one-way analysis of variance (ANOVA). When significant treatment effects were detected, mean values were compared using Tukey's honestly significant difference (HSD) test. Differences were considered statistically significant at $p < 0.05$ (p is italicized).

3. Results

3.1. Isolation and Identification of Phytopathogenic Fungi and Antagonistic Activity of Putative Nitrogen-Fixing Bacteria

The isolated fungal cultures exhibited distinct cultural and morphological characteristics, including differences in colony appearance and microscopic structures such as conidia and conidiophores. Representative morphological characteristics of the isolated *Fusarium* and *Alternaria* fungi are presented in Figure 2.

Two fungal isolates were selected for the antagonistic assays following morphological and ITS-based molecular characterization. Isolate 5PS was assigned to *Fusarium proliferatum*, whereas isolate 6PS was assigned to *Alternaria alternata* based on the combined morphological, BLAST, and phylogenetic evidence. These isolates were subsequently used in the dual-culture antagonistic assays (Table 1).

Table 1. Molecular identification of phytopathogenic fungal isolates based on sequence analysis.

Isolate	Identification	GenBank Accession No.
5PS	*Fusarium proliferatum*	MG274295.1
6PS	*Alternaria alternata*	MK972909.1

To further confirm the taxonomic position of the isolates, phylogenetic analysis was performed using nucleotide sequence data. Phylogenetic analysis based on ITS sequences further supported the identification of the fungal isolates. Isolate 5PS was positioned within

the *Fusarium proliferatum* group, while isolate 6PS was placed within the *Alternaria* clade together with closely related reference sequences (Figure 3).

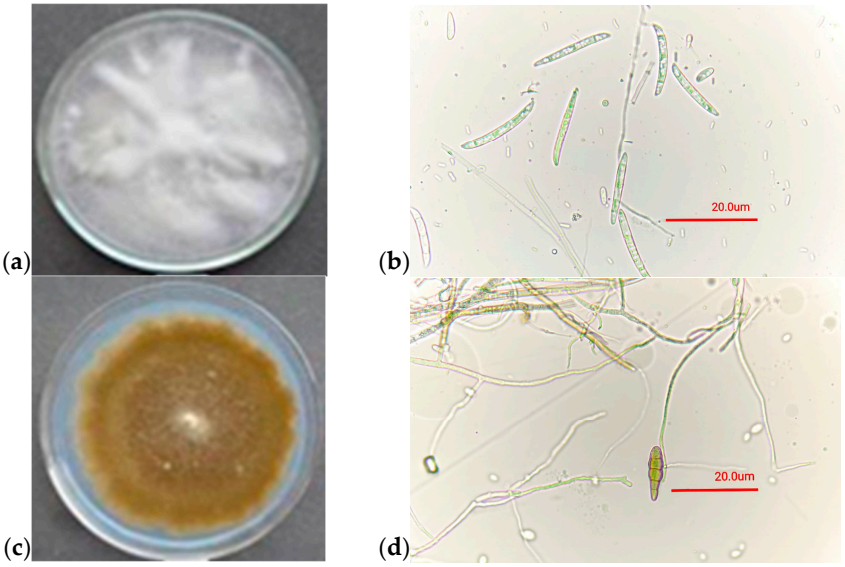

Figure 2. Morphological characteristics of fungal isolates obtained from infected cereal crop samples. (**a**) Colony morphology of *Fusarium*. (**b**) Macroconidia of the *Fusarium* isolate. Scale bar = 20 µm. (**c**) Colony morphology of *Alternaria*. (**d**) Conidia of *Alternaria.* Scale bar = 20 µm.

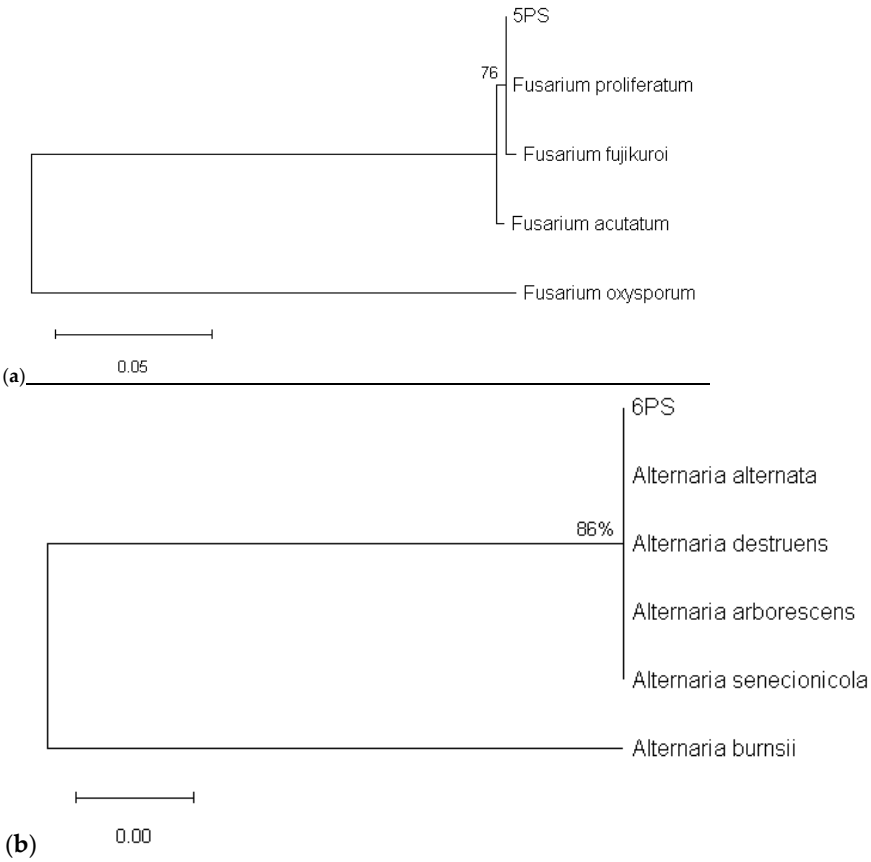

Figure 3. Neighbor-Joining phylogenetic trees constructed from ITS region sequences of representative fungal isolates. (**a**) Phylogenetic relationships of the *Fusarium* isolate 5PS with reference sequences. (**b**) Phylogenetic relationships of *Alternaria* isolates, including isolate 6PS, with reference sequences. The scale bar represents 0.01 substitutions per site. Bootstrap values (%) are indicated at the corresponding nodes.

The molecular identification results for the bacterial isolates based on 16S rRNA gene sequences are presented in Table 2.

Table 2. Molecular identification of bacterial isolates based on 16S rRNA gene sequences.

Isolate	Bacterial Identification	GenBank Accession No.
2 S	*Rhizobium giardinii*	PZ963067
3S	*Alphaproteobacteria bacterium*	Not available
6S	*Agrobacterium fabrum*	PZ963068
7S	*Agrobacterium tumefaciens*	Not available
8S	*Sphingobium yanoikuyae*	PZ963069
9S	*Synergistetes bacterium*	Not available
10S	*Rhizobium radiobacter*	Not available
11S	*Streptomyces virginiae*	Not available
20S	*Brevundimonas bullata*	PZ963070
21S	*Hansschlegelia plantiphila*	PZ963071
35S	*Priestia megaterium*	PZ963072
49S	*Sphingobium yanoikuyae*	PZ963073
51S	*Agrobacterium pusense*	PZ963074
52S	*Agrobacterium tumefaciens*	PZ963075
53S	*Brevundimonas bullata*	PZ963076
54S	*Agrobacterium tumefaciens*	PZ963077
55S	*Rhizobium pusense*	Not available
56S	*Ensifer meliloti*	PZ963078
57S	*Arthrobacter pascens*	Not available
59S	*Ensifer fredii*	PZ963079
65S	*Paenibacillus taichungensis*	PZ963080
69S	*Brevibacterium sediminis*	PZ963081
70S	*Oricola cellulosilytica*	PZ963082
81S	*Rhizobium radiobacter*	PZ963083
84S	*Actinomycetes bacterium*	PZ963084
91S	*Ensifer* sp.	PZ963085

The 16S rRNA gene sequences of all 26 bacterial isolates were analyzed for molecular identification. The identified bacterial isolates and the corresponding GenBank accession numbers are presented in Table 2. Accession numbers were obtained for 19 isolates, whereas accession numbers for the remaining seven isolates are currently unavailable.

The phylogenetic relationships among the bacterial isolates were further evaluated based on their 16S rRNA gene sequences. The resulting Neighbor-Joining phylogenetic tree provides a visual representation of the taxonomic relationships of the bacterial isolates analyzed in this study (Figure 4).

A total of 26 putative nitrogen-fixing bacterial isolates were evaluated for their antagonistic activity against *Fusarium proliferatum* and *Alternaria alternata* using the dual-culture assay. Considerable variation in antifungal activity was observed among the tested isolates. The inhibition zones ranged from 0 to 30 mm, indicating substantial differences in the biocontrol potential of the bacterial strains (Table 3).

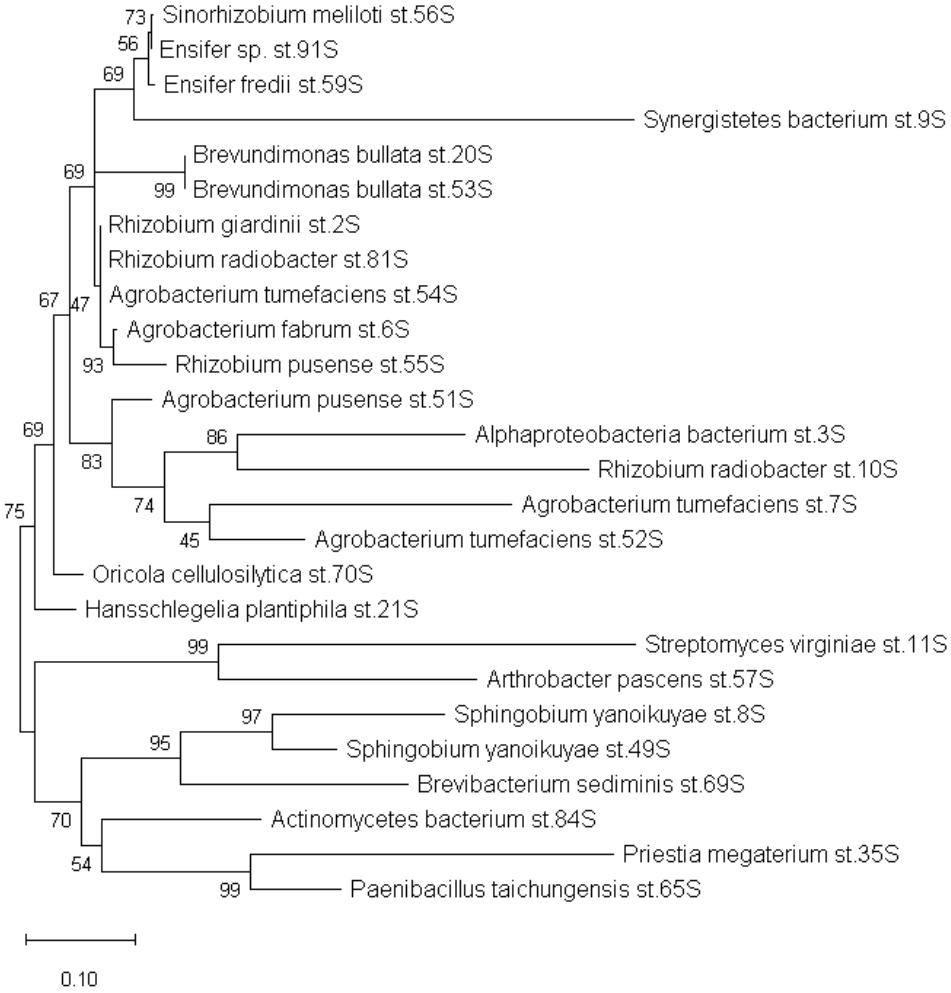

Figure 4. Neighbor-Joining phylogenetic tree constructed from 16S rRNA gene sequences of bacterial isolates. The tree illustrates the phylogenetic relationships between the bacterial isolates obtained in this study and closely related reference sequences. Bootstrap values (%) are indicated at the corresponding nodes.

Table 3. Antagonistic activity of putative nitrogen-fixing bacterial strains against *Fusarium proliferatum* and *Alternaria alternata*.

Strain №	**Inhibition Zone, mm**	
	Fusarium proliferatum	***Alternaria alternata***
Rhizobium giardinii st.2S	0 h	10 ± 2.0 fg
Alphaproteobacteria bacterium st.3S	20 ± 2.0 bcdef	9 ± 2.0 fg
Agrobacterium fabrum st.6S	20 ± 1.7 bcde	9 ± 3.6 g
Agrobacterium tumefaciens st.7S	9 ± 1.0 fgh	5 ± 2.0 gh
Sphingobium yanoikuyae st.8S	15 ± 2.0 cdef	0 h
Synergistetes bacterium st.9S	25 ± 2.6 bc	25 ± 2.1 abc
Rhizobium radiobacter st.10S	13 ± 3.7 defg	17 ± 1.7 def
Streptomyces virginiae st.11S	25 ± 4.4 bc	22 ± 3.6 bcd
Brevundimonas bullata st.20S	10 ± 2.6 efgh	17 ± 2.6 def
Hansschlegelia plantiphila st.21S	25 ± 2.6 bc	13 ± 2.1 efg
Priestia megaterium st.35S	21 ± 3.6 bcd	9 ± 1.7 g

Table 3. *Cont.*

Strain №	Inhibition Zone, mm	
	Fusarium proliferatum	***Alternaria alternata***
Sphingobium yanoikuyae st.49S	0 h	0 h
Agrobacterium pusense st.51S	30 ± 3.6 ab	25 ± 2.6 abc
Agrobacterium tumefaciens st.52S	15 ± 3.6 cdef	10 ± 0.6 fg
Brevundimonas bullata st.53S	28 ± 3.6 ab	9 ± 2.6 g
Agrobacterium tumefaciens st.54S	22 ± 3.6 bcd	10 ± 2.6 fg
Rhizobium pusense st.55S	10 ± 3.6 efgh	25 ± 3.0 abc
Ensifer meliloti st.56S	25 ± 3.6 bc	20 ± 2.6 cde
Arthrobacter pascens st.57S	20 ± 3.7 bcde	7 ± 2.0 gh
Ensifer fredii st.59S	30 ± 4.7 ab	5 ± 1.7 gh
Paenibacillus taichungensis st.65S	3 ± 2.6 gh	10 ± 2.6 fg
Brevibacterium sediminis st.69S	15 ± 4.3 cdef	17 ± 2.6 def
Oricola cellulosilytica st.70S	30 ± 2.6 ab	30 ± 3.6 a
Rhizobium radiobacter st.81S	25 ± 3.6 bc	20 ± 1.7 cde
Actinomycetes bacterium st.84S	20 ± 2.6 bcde	28 ± 2.6 ab
Ensifer sp. st.91S	22 ± 2.6 bcd	12 ± 3.1 efg

Data are presented as mean ± SD. Different lowercase letters within a column indicate statistically significant differences according to one-way analysis of variance (ANOVA) followed by Tukey's HSD test at $p < 0.05$.

The strongest inhibition of *Fusarium proliferatum* was observed for *Agrobacterium pusense* st.51S, *Ensifer fredii* st.59S, and *Oricola cellulosilytica* st.70S. The highest inhibitory activity against *Alternaria alternata* was recorded for *Oricola cellulosilytica* st.70S, *Synergistetes bacterium* st.9S, *Agrobacterium pusense* st.51S, and *Rhizobium pusense* st.55S. In addition, *O. cellulosilytica* st.70S, *Streptomyces virginiae* st.11S, and *Ensifer meliloti* st.56S inhibited the growth of both tested phytopathogenic fungi.

Overall, the obtained results demonstrate that several indigenous bacterial isolates from Northern Kazakhstan possess antagonistic activity against economically important cereal phytopathogens. These findings support their potential application as environmentally friendly biological control agents capable of reducing disease pressure while contributing to sustainable crop management.

3.2. Effect of Putative Nitrogen-Fixing Bacteria on the In Vitro Germination of Buckwheat Seeds

The analysis of the effects of bacterial strain culture filtrates on the germination and early development of the buckwheat variety 'Shortandinskaya 6' revealed significant treatment effects across the evaluated parameters (Table 4).

For the variety 'Shortandinskaya 6', treatment with the culture filtrates of *Ensifer meliloti* st.56S, *Paenibacillus taichungensis* st.65S, *Brevundimonas bullata* st.20S, *Rhizobium radiobacter* st.10S, and *Agrobacterium fabrum* st.6S resulted in a statistically significant increase in shoot and root length compared with the control. The greatest effect was observed for *E. meliloti* st.56S, which increased shoot and root length by 31.4% and 23.8%, respectively. The highest germination energy and germination percentage were obtained following treatment with *Ensifer* sp. st.91S, with values significantly exceeding those of the control.

Table 4. Effects of Microbial Culture Filtrates on the Growth and Development of the Buckwheat variety 'Shortandinskaya 6'.

Strain №	Germination Energy, % (Day 3)	Germination, % (Day 7)	Shoot Length, cm	Root Length, cm
1	2	3	4	5
Control	86.7 ± 2.9 ab	88.3 ± 2.9 ab	5.1 ± 0 ef	6.3 ± 0 hi
Rhizobium giardinii st.2S	88.3 ± 2.9 ab	93.3 ± 2.9 ab	5.0 ± 0.1 f	6.1 ± 0.1 i
Alphaproteobacteria bacterium st.3S	86.7 ± 2.9 ab	88.3 ± 2.9 ab	5.4 ± 0.3 cdef	6.7 ± 0.3 defghi
Agrobacterium fabrum st.6S	85.5 ± 5.0 ab	90.0 ± 5.0 ab	6.3 ± 0.4 abc	7.6 ± 0.2 abc
Agrobacterium tumefaciens st.7S	86.7 ± 2.9 ab	91.7 ± 2.9 ab	6.4 ± 0.4 ab	7.5 ± 0.4 abcde
Sphingobium yanoikuyae st.8S	88.3 ± 2.9 ab	91.7 ± 2.9 ab	6.2 ± 0.1 abc	7.3 ± 0.1 abcdef
Synergistetes bacterium st.9S	85.0 ± 5.0 ab	86.7 ± 2.9 b	5.5 ± 0.2 cdef	6.7 ± 0.3 fghi
Rhizobium radiobacter st.10S	93.3 ± 2.9 ab	96.7 ± 5.8 ab	6.5 ± 0.3 a	7.5 ± 0.2 abcd
Streptomyces virginiae st.11S	91.7 ± 2.9 ab	96.7 ± 2.9 ab	5.9 ± 0.1 abcde	6.7 ± 0.2 efghi
Brevundimonas bullata st.20S	91.7 ± 2.9 ab	96.7 ± 2.9 ab	6.5 ± 0.5 a	7.7 ± 0.5 ab
Hansschlegelia plantiphila st.21S	86.7 ± 2.9 ab	91.7 ± 2.9 ab	6.1 ± 0.4 abcd	7.2 ± 0.4 abcdefg
Priestia megaterium st.35S	91.7 ± 2.9 ab	93.3 ± 2.9 ab	6.3 ± 0.3 abc	7.6 ± 0.2 abc
Sphingobium yanoikuyae st.49S	83.3 ± 2.9 b	88.3 ± 2.9 ab	6.1 ± 0.2 abcd	7.4 ± 0.1 abcdef
Agrobacterium pusense st.51S	88.3 ± 5.8 ab	91.7 ± 2.9 ab	5.2 ± 0.1 ef	6.4 ± 0.1 hi
Agrobacterium tumefaciens st.52S	86.7 ± 2.9 ab	91.7 ± 2.9 ab	5.0 ± 0.2 f	6.2 ± 0.2 i
Brevundimonas bullata st.53S	83.3 ± 5.8 b	88.3 ± 5.8 ab	5.0 ± 0.2 f	6.3 ± 0.1 hi
Agrobacterium tumefaciens st.54S	91.7 ± 2.9 ab	91.7 ± 2.9 ab	5.1 ± 0.2 ef	6.2 ± 0.2 i
Rhizobium pusense st.55S	91.7 ± 7.6 ab	95.0 ± 5.0 ab	5.0 ± 0.2 f	6.1 ± 0.2 i
Ensifer meliloti st.56S	86.7 ± 2.9 ab	93.3 ± 2.9 ab	6.7 ± 0.2 a	7.8 ± 0.2 a
Arthrobacter pascens st.57S	86.7 ± 2.9 ab	91.7 ± 2.9 ab	5.6 ± 0.2 cdef	6.7 ± 0.2 fghi
Ensifer fredii st.59S	93.3 ± 5.8 ab	96.7 ± 2.9 ab	5.3 ± 0.1 def	6.5 ± 0.2 ghi
Paenibacillus taichungensis st.65S	91.7 ± 2.9 ab	93.3 ± 2.9 ab	6.6 ± 0.3 a	7.7 ± 0.3 ab
Brevibacterium sediminis st.69S	83.3 ± 2.9 b	91.7 ± 2.9 ab	6.3 ± 0.1 abc	7.3 ± 0.1 abcdef
Oricola cellulosilytica st.70S	91.7 ± 2.9 ab	96.7 ± 2.9 ab	6.1 ± 0.3 abcd	7.2 ± 0.3 abcdefg
Rhizobium radiobacter st.81S	88.3 ± 2.9 ab	93.3 ± 2.9 ab	5.9 ± 0.1 abcde	7.0 ± 0.1 bcdefgh
Actinomycetes bacterium st.84S	83.3 ± 2.9 b	91.7 ± 2.9 ab	6.2 ± 0.3 abc	7.3 ± 0.3 abcdef
Ensifer sp. st.91S	96.7 ± 2.9 a	98.3 ± 2.9 a	6.1 ± 0.3 abcd	7.3 ± 0.3 abcdef

Data are presented as mean ± SD. Different lowercase letters within a column indicate statistically significant differences according to one-way analysis of variance (ANOVA) followed by Tukey's HSD test at $p < 0.05$.

In the variety 'Shortandinskaya Krupnozernaya', the response to treatment was less pronounced (Table 5).

Table 5. Effects of Microbial Culture Filtrates on the Growth and Development of the Buckwheat variety 'Shortandinskaya Krupnozernaya'.

Strain №	Germination Energy, % (Day 3)	Germination, % (Day 7)	Shoot Length, cm	Root Length, cm
1	2	3	4	5
Control	92.3 ± 2.5 b	93.3 ± 2.9 bcd	5.3 ± 0.2 bc	6.6 ± 0.3 bcde
Rhizobium giardinii st.2S	94.0 ± 1.7 ab	98.3 ± 2.9 a	5.6 ± 0.1 abc	6.7 ± 0.4 bcde
Alphaproteobacteria bacterium st.3S	92.3 ± 2.5 ab	95.3 ± 0.6 abcd	5.3 ± 0.3 bc	6.7 ± 0.4 bcde
Agrobacterium fabrum st.6S	94.0 ± 1.7 ab	96.7 ± 2.9 abc	5.4 ± 0.3 bc	6.6 ± 0.3 cde
Agrobacterium tumefaciens st.7S	91.7 ± 2.9 b	92.3 ± 2.5 cd	5.3 ± 0.3 bc	6.6 ± 0.4 bcde
Sphingobium yanoikuyae st.8S	92.3 ± 2.5 b	93.7 ± 3.2 bcd	5.3 ± 0.5 bc	6.7 ± 0.6 bcde
Synergistetes bacterium st.9S	91.7 ± 2.9 b	93.7 ± 3.2 bcd	5.2 ± 0.4 c	6.4 ± 0.5 de

Table 5. *Cont.*

Strain №	Germination Energy, % (Day 3)	Germination, % (Day 7)	Shoot Length, cm	Root Length, cm
Rhizobium radiobacter st.10S	92.3 ± 2.5 b	95.3 ± 0.6 abcd	5.1 ± 0.2 c	6.3 ± 0.2 e
Streptomyces virginiae st.11S	91.3 ± 1.2 b	93.7 ± 3.2 bcd	5.2 ± 0.3 bc	6.5 ± 0.2 cde
Brevundimonas bullata st.20S	93.3 ± 2.9 ab	97.0 ± 2.6 ab	5.2 ± 0.4 c	6.6 ± 0.2 bcde
Hansschlegelia plantiphila st.21S	93.3 ± 2.9 ab	95.3 ± 0.6 abcd	5.3 ± 0.1 bc	6.8 ± 0.3 bcde
Priestia megaterium st.35S	96.7 ± 2.9 a	98.3 ± 2.9 a	5.5 ± 0.3 abc	6.9 ± 0.5 bcde
Sphingobium yanoikuyae st.49S	91.7 ± 2.9 b	93.3 ± 2.9 bcd	5.3 ± 0.5 bc	6.6 ± 0.5 bcde
Agrobacterium pusense st.51S	91.7 ± 2.9 b	94.0 ± 1.7 abcd	5.2 ± 0.3 c	6.5 ± 0.3 cde
Agrobacterium tumefaciens st.52S	90.7 ± 1.2 b	92.0 ± 3.5 d	5.4 ± 0.3 bc	6.7 ± 0.2 bcde
Brevundimonas bullata st.53S	91.7 ± 2.9 b	93.7 ± 3.2 bcd	5.3 ± 0.5 bc	6.7 ± 0.5 bcde
Agrobacterium tumefaciens st.54S	94.0 ± 1.7 ab	95.3 ± 0.6 abcd	5.4 ± 0.2 bc	6.8 ± 0.1 bcde
Rhizobium pusense st.55S	93.3 ± 2.9 ab	95.7 ± 4.0 abcd	5.7 ± 0.4 abc	7.0 ± 0.5 abcde
Ensifer meliloti st.56S	93.7 ± 3.2 ab	96.7 ± 2.9 abc	5.7 ± 0.3 abc	7.2 ± 0.3 abcd
Arthrobacter pascens st.57S	91.7 ± 2.9 b	92.0 ± 3.5 d	5.4 ± 0.5 bc	6.9 ± 0.7 bcde
Ensifer fredii st.59S	91.7 ± 2.9 b	96.7 ± 2.9 abc	5.7 ± 0.2 abc	7.2 ± 0.3 abc
Paenibacillus taichungensis st.65S	93.3 ± 2.9 ab	95.3 ± 0.6 abcd	5.4 ± 0.6 bc	6.9 ± 0.4 bcde
Brevibacterium sediminis st.69S	91.7 ± 2.9 b	93.3 ± 2.9 bcd	5.6 ± 0.8 abc	7.1 ± 0.7 abcde
Oricola cellulosilytica st.70S	91.7 ± 2.9 b	93.7 ± 3.2 bcd	5.6 ± 0.3 abc	7.2 ± 0.4 abcd
Rhizobium radiobacter st.81S	90.7 ± 1.2 ab	93.3 ± 2.9 bcd	5.9 ± 0.7 c	7.4 ± 0.8 bcde
Actinomycetes bacterium st.84S	93.3 ± 2.9 b	93.3 ± 2.9 bcd	5.2 ± 0.9 ab	6.7 ± 1.0 ab
Ensifer sp. st.91S	91.7 ± 2.9 b	95.3 ± 0.6 abcd	6.2 ± 0.9 a	7.7 ± 0.9 a

Data are presented as mean ± SD. Different lowercase letters within a column indicate statistically significant differences according to one-way analysis of variance (ANOVA) followed by Tukey's HSD test at $p < 0.05$.

A significant increase in germination energy and germination percentage was observed following treatment with *Priestia megaterium* st.35S and *Rhizobium giardinii* st.2S. The greatest improvement in biometric traits was obtained with *Ensifer* sp. st.91S, which increased shoot and root length by 17.0% and 16.7%, respectively. The strains that exhibited the most pronounced and statistically validated beneficial effects will be used in the further selection of promising components for the development of a bioformulation for the pre-sowing treatment of buckwheat seeds.

4. Discussion

Fungal diseases remain one of the major constraints limiting cereal production worldwide. Species belonging to the genera *Fusarium* and *Alternaria* are among the most destructive phytopathogens because of their ability to survive in soil and infected plant residues, colonize seeds, and cause significant yield and quality losses [36]. Therefore, accurate identification of these pathogens is essential for developing effective biological control strategies. *Fusarium proliferatum* is a globally distributed phytopathogenic fungus associated with numerous economically important crops. In addition to causing root and stem diseases, it can be transmitted through infected seeds and is recognized as an important producer of mycotoxins that reduce grain quality and pose risks to food safety. Likewise, *Alternaria alternata* is one of the most widespread fungal pathogens affecting cereal and horticultural crops, causing leaf spot diseases and seed contamination under a wide range of environmental conditions.

In addition to their plant growth-promoting properties, the putative nitrogen-fixing bacteria investigated in this study demonstrated antagonistic activity against the phytopathogenic fungi *Fusarium proliferatum* and *Alternaria alternata*. Several strains inhibited

fungal growth, while *Agrobacterium pusense* st.51S, *Ensifer fredii* st.59S, and *Oricola cellulosilytica* st.70S showed the highest inhibitory activity against *F. proliferatum* among the tested strains. The ability of *O. cellulosilytica* st.70S, *Streptomyces virginiae* st.11S, and *Ensifer meliloti* st.56S to suppress both fungal pathogens suggests that some of the tested bacteria may possess a broader spectrum of biocontrol activity.

The observed antagonistic responses were pathogen-specific, with some strains effectively suppressing one fungal species while showing little or no activity against the other. Such differences may reflect variations in the spectrum of antimicrobial metabolites, lytic enzymes, volatile organic compounds, or siderophores produced by individual bacterial strains. Similar pathogen-specific antagonistic responses have been reported for other plant growth-promoting rhizobacteria and are attributed to differences in fungal cell wall composition and susceptibility to bacterial metabolites.

The observed antagonistic effects may be associated with mechanisms commonly reported for beneficial bacteria, including the production of antimicrobial compounds, hydrolytic enzymes, siderophores, and other metabolites, as well as competition for nutrients and ecological niches. However, these mechanisms were not directly investigated in the present study and therefore cannot be confirmed for the strains tested here. Similar mechanisms have been described for plant growth-promoting bacteria, which can simultaneously promote plant development and reduce the activity of soil- and seed-associated pathogens [37,38]. The stronger inhibition observed for some strains against *F. proliferatum* compared with *A. alternata* may reflect differences in fungal sensitivity to bacterial metabolites and in the physiological characteristics of the pathogens.

The present findings demonstrate that putative nitrogen-fixing bacteria possess considerable potential as plant growth promoters and biocontrol agents [39], in agreement with previous studies. The ability of microorganisms to stimulate plant growth has been widely documented. Significant improvements in seed germination and root establishment have been reported for various crops following seed treatment with plant growth-promoting strains. For example, inoculation with *Bacillus cereus* and *Sinorhizobium meliloti* increased the germination percentage and germination index of cucumber by 20–60% relative to the control, while shoot and root lengths increased by up to 48% and 11%, respectively [34].

Similar responses have been observed in cereal crops. Seed treatment of wheat with *Azotobacter chroococcum* and *Azospirillum lipoferum* significantly enhanced both germination and root length compared with the control [40]. In addition, studies involving *Streptomyces* spp. in wheat reported increases in root length by 1.7–1.8-fold and shoot length by up to 3.3-fold following inoculation with selected strains, highlighting the strong growth-promoting potential of these bacteria [41].

In the present study, culture filtrates of several strains exhibited pronounced growth-stimulatory effects. The most pronounced enhancement of germination energy and germination percentage was observed for *Rhizobium radiobacter* strain 10S and *Ensifer* sp. st.91S, respectively. These effects are consistent with those reported in international studies, in which plant growth-promoting rhizobacteria enhanced root growth and seedling development in cereal crops [42].

Such responses may be associated with the production of phytohormones, particularly indole-3-acetic acid (IAA), as well as other growth regulators and microbial metabolites that can influence nutrient availability [43]. However, the production of IAA, siderophores, hydrolytic enzymes, or other specific bioactive metabolites was not directly measured in the present study. Therefore, these mechanisms should be regarded as possible explanations rather than experimentally confirmed mechanisms underlying the observed effects. This interpretation is consistent with our observation of increased root number and root length in the treated variants relative to the control, indicating more active root development and

potentially improved nutrient acquisition. Recent studies have highlighted the diverse roles of beneficial plant-associated microorganisms in regulating plant growth, nutrient acquisition, stress responses, and pathogen resistance [44–46]. Further characterization of the most promising strains will be required to determine the specific metabolites and mechanisms responsible for their plant growth-promoting effects.

The observed effects were, however, strain-dependent. Some strains in previous studies exhibited weaker responses or even growth inhibition under specific conditions, which is consistent with our findings for several strains that did not differ significantly from the control [41]. Such variability may be associated with differences in bacterial metabolic activity, production of phytohormones and other metabolites, compatibility with the host plant genotype, and environmental conditions. Therefore, the selection of effective strains is an important step in the development of microbial preparations for agricultural application.

The responses of the two buckwheat varieties also indicate that the effectiveness of bacterial treatments may depend on plant genotype. Differences in the response of 'Shortandinskaya 6' and 'Shortandinskaya Krupnozernaya' suggest that bacterial–plant interactions are not uniform and should be considered when selecting strains for practical application. The pronounced response of 'Shortandinskaya 6' to *E. meliloti* st.56S and the positive effects of *Ensifer* sp. st.91S and *Priestia megaterium* st.35S on 'Shortandinskaya Krupnozernaya' indicate that strain–variety compatibility may be an important factor determining the effectiveness of microbial inoculation. These findings indicate that the effects of microbial metabolites are variety-specific and may depend on the interaction between bacterial strains and the plant genotype.

The combined growth-promoting and antagonistic properties observed for several strains are particularly relevant for the development of multifunctional microbial preparations. Such multifunctional strains combine plant growth-promoting potential with biological control activity, making them promising candidates for the development of microbial bioformulations for sustainable crop production. Instead of acting exclusively as biofertilizers or biological control agents, such microorganisms may simultaneously contribute to improved seedling establishment and suppression of phytopathogenic fungi. This multifunctionality is especially important for sustainable crop production, where reducing dependence on synthetic fertilizers and chemical plant protection products is increasingly desirable.

Overall, comparison of our results with international data indicates that the putative nitrogen-fixing bacterial strains investigated in this study can enhance seed germination, shoot elongation, and root development, while some strains also exhibit antagonistic activity against phytopathogenic fungi. The magnitude of the growth-promoting effects observed in the present study falls within the range reported elsewhere [47]. The identification of locally adapted strains with both plant growth-promoting and biocontrol properties provides a promising basis for the development of multifunctional microbial bioformulations for buckwheat production under the conditions of Northern Kazakhstan. Further studies should focus on optimization of formulation, strain compatibility, mechanisms of action, and validation under field conditions.

Limitations of the study. The present study was conducted under the specific soil and climatic conditions of Northern Kazakhstan, which may limit the direct extrapolation of the obtained results to other agroecological regions. In addition, the field experiment covered a limited growing period, and therefore, the long-term effects of the tested nitrogen-fixing biopreparations on soil fertility and buckwheat productivity require further investigation. The effectiveness of microbial inoculants may also vary depending on soil properties, weather conditions, crop genotype, and the composition of the indigenous rhizosphere

microbiota. Further multi-year and multi-location studies are therefore needed to confirm the stability and broader applicability of the observed effects.

Additional limitations include the absence of an external positive control in the dual-culture assay and the lack of direct measurements of the metabolites potentially responsible for the observed plant growth-promoting and antagonistic effects. Therefore, the present findings should be considered a screening-level assessment of strain performance. Future studies should include appropriate positive controls and direct characterization of the relevant microbial metabolites and mechanisms of action.

An additional limitation is that an uninoculated Ashby's medium control was not included in the seed germination assay. The control treatment consisted of sterile distilled water; therefore, the possibility that residual components of the growth medium contributed to the observed germination responses cannot be completely excluded. Future experiments should include an uninoculated medium control to distinguish the effects of bacterial metabolites from those of the growth medium itself.

5. Conclusions

The present study demonstrated that indigenous putative nitrogen-fixing bacteria isolated from the dry steppe zone of Northern Kazakhstan possess both plant growth-promoting and biocontrol properties. Several strains showed antagonistic activity against *Fusarium proliferatum* and *Alternaria alternata*, while seed treatment with bacterial culture filtrates improved germination and early seedling growth of the buckwheat varieties 'Shortandinskaya 6' and 'Shortandinskaya Krupnozernaya'. In particular, *Ensifer meliloti* st.56S increased shoot and root length in 'Shortandinskaya 6' by 31.4% and 23.8%, respectively, while *Ensifer* sp. st.91S and *Priestia megaterium* st.35S demonstrated pronounced positive effects on seed germination and root development. These findings indicate the potential of indigenous putative nitrogen-fixing bacteria as promising candidates for the development of multifunctional microbial bioformulations. The significance of the study lies in identifying locally adapted bacterial strains that may serve as a biological basis for developing environmentally friendly biofertilizers aimed at improving buckwheat establishment, reducing the impact of phytopathogens, and supporting sustainable crop production under the conditions of Northern Kazakhstan. Further studies should focus on the formulation, compatibility, and field-scale evaluation of the most effective strains.

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Data Availability Statement: The 16S rRNA gene sequences of 19 bacterial isolates generated in this study have been deposited in the NCBI GenBank database under accession numbers PZ963067–PZ963085. The 16S rRNA gene sequences of the remaining seven bacterial isolates were analyzed for molecular identification, but GenBank accession numbers are currently unavailable. All other data generated or analyzed during the study are included in the article.

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Abbreviations

The following abbreviations are used in this manuscript

PGPR	Plant Growth-Promoting Rhizobacteria
N	Nitrogen
IAA	Indole-3-acetic acid
mm	Millimeter
PCR	Polymerase Chain Reaction
rRNA	Ribosomal Ribonucleic Acid
ITS	Internal Transcribed Spacer
BLAST	Basic Local Alignment Search Tool
NJ	Neighbor-Joining
SD	Standard Deviation
bp	Base Pair
HSD	Honestly Significant Difference

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