Original Article

Creating a biological product using Nitrogen-fixing bacteria before sowing wheat (north Kazakhstan)

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ABSTRACT

Based on the associations of nitrogen-fixing bacteria of the *Rhizobium sp.* and *Azotobacter sp.* genera, biofertilizers have been developed for increasing the wheat yield in Northern Kazakhstan. Among nodule bacteria and azotobacters, nine promising strains of microorganisms that can stimulate plant growth, fix nitrogen, and exhibit antagonism to phytopathogenic micromycetes have been isolated. Three associations have been tested in the vegetation experiments. According to the results of the experiment, association No. 2 that was tested in field studies upon introduction before sowing spring wheat was selected. The biofertilizer has increased the wheat grain yield by 275 kg/ha.

Keywords: Biofertilizer, Nitrogen-fixing bacteria, Nodule bacteria, Biocompatibility, Antifungal activity, Field experiment

Introduction

Kazakhstan has a significant potential for developing organic production due to the low level of using mineral fertilizers and pesticides and the availability of significant areas of fertile chernozem soil in the north of the country. The agro-industrial complex of Kazakhstan can become one of the world's largest producers of agricultural export products, especially organic food. All necessary political, legal, and scientific prerequisites for organic farming development are in place. For instance, within the framework of the Concept for the transition to a green economy, the law "On the production of organic products" has been adopted, the Organic Center is operating under the aegis of the Ministry of Agriculture of the Republic of Kazakhstan, technologies of biological farming are being developed, and the interest of agricultural companies to organic production is growing due to the higher price of organic products. The demand for ecologically clean food is growing 1.5 – 2 times faster than the supply, which gives Kazakhstan great opportunities due to the availability of clean land [1].

Organic farming is based on reducing or completely weaning off the use of synthetic mineral fertilizers, plant protection chemicals, plant growth regulators, and genetically modified organisms, and the maximum use of biological products and biotechnologies at all stages of agricultural production [2].

Complex biological products that combine the properties and functions of various biological products are preferable [3].

Practice shows that the use of complex biofertilizers contributes to a stable yield growth by 25 – 50% and more with significantly reduced root rot of the plants [4, 5]. The fungistatic effect is usually there because the level of soil biodiversity grows significantly, while the importance of unwanted populations (for example, phytopathogenic fungi) decreases.

The meta-analysis of the data about the biological products intended for agriculture showed the advantage and effectiveness of biofertilizers based on nitrogen-fixing and phosphate-mobilizing bacteria [6].

In the scientific literature, there are several reports about attempts to create biofertilizers based on associations of nitrogen-fixing bacteria of the *Rhizobium* and *Azotobacter* genera. For instance, Korniichuk *et al.* claimed to develop a promising biofilm biofertilizer from the free-living bacteria of the *Rhizobium*

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and *Azotobacter* genera, which positively influenced the mass of lettuce sprouts, providing a 49.6% increase in the biomass, compared to untreated reference lettuce. The positive effect was due to the nature of the bacteria of the PGPR group [7].

An important property of the *Azotobacter* genus is the ability of some species to degrade environmental pollutants. It should be noted that the *Azotobacter* bacteria combine well with the bacteria of the *Clostridium, Pseudomonas, Bacillus, Azospirillum, Agrobacterium* genera, and, most importantly, rhizosphere bacteria (the *Rhizobiaceae* family) [8].

For Kazakhstan, the most important crop is spring wheat. The concept of developing a green economy in the Republic of Kazakhstan sets the task of significantly increasing the yield of this crop. Biofertilizers and biopesticides can play an important role in accomplishing this task [9].

In this work, the goal was developing a nitrogen-fixing bacteria-based biofertilizer for wheat that would be suitable for Northern Kazakhstan.

Materials and Methods

Soil samples were taken using the envelope method from the rhizosphere of plants in the crops of wheat, corn, lentils, and peas of LLP Agricultural Experimental Station Zarechnoye, and the Dostyk peasant farm. The pure cultures of the bacteria of the *Azotobacter* genus were isolated from the soil using the method of lumps according to Vinogradsky [10]. Nodule bacteria were isolated from the lentil nodules selected in the flowering phase from large and dense nodules.

The objects of the study were *Rhizobium leguminosarum* nitrogen-fixing nodule bacteria, Rh-1, Rh-2, Rh-5 strains, and bacteria of the *Azotobacter* sp. genus, Az-34, Az-4, Az-6, Az-20, Az-28, Az-13, Az-1/2 strains, obtained by the authors earlier from the rhizosphere of agricultural plants. Nitrogen-fixing bacteria were grown on Burk's medium, while nodule bacteria — in the agar medium with bean broth.

Nitrogen fixation of the bacteria was determined by the accumulation of ammonium in the medium [11]. The nitrogen-fixing activity of the isolated strains was determined based on measuring the concentration of free NH_4^+ ions in the culture liquid.

The biofertilizer was tested upon sowing spring wheat of the Omskaya-36 variety.

Chemical fungicide Lamador manufactured by Bayer Schering Pharma, USA was used for seed treatment.

The antifungal activity of the bacteria to *Fusarium sp., Alternaria alternata* was determined using the method of wells in the joint cultivation of the bacteria and phytopathogens on the Sabouraud and Chapek-3 media [12].

The strains were identified by determining the direct nucleotide sequence of the 16S rRNA gene fragment, followed by comparing the nucleotide identity with the sequences deposited in the international GeneBank database [13].

To study the effect of liquid preparation forms of bacterial fertilizers on the growth of grain crops, a vegetation experiment was laid in a greenhouse of the National Center for Biotechnology in Nur-Sultan.

Field experiments were laid on a fallow field, the soil was southern chernozem. The field was prepared and the experiments were laid as per the relevant recommendations [14], with separate additions and changes adopted at LLP Agricultural Experimental Station Zarechnoye**,** the soil in the fallow field was southern chernozem.

Observation and accounting, land plot selection and preparation, laying and performing the experiment, and statistical processing of the results were performed according to Dospechov [15].

The percentage of protein, gluten, and moisture was measured using an IR analyzer at an ambient temperature of 21 °C (18 – 25) and relative humidity of 64% (30-80%) [16]. The quantitative protein content was determined following the state industry standard. For the mathematical processing of the results, standard methods for finding mean values and their mean errors were used [17].

Results and Discussion

Studying the cultural properties of the strains of nitrogen-fixing bacteria

The morphological and biochemical properties of nine selected strains of azotobacter and nodule bacteria were studied. Following the cultural and phenotypic properties, six strains (Az-4/3, Az13, Az-6, Az-2/1, Az-9/2, and Az-34) were assigned to the *Azotobacter* genus according to Bergey's Manual. The strains produced a water-insoluble brown pigment, had peritrichous flagella and formed cysts. On Ashby's medium, the strains formed large, round, convex, and slimy colonies of 7 to 13 mm in diameter with a brown pigment and smooth edges. With aging, the colonies became slimmer and spread over the agar surface.

The strains of nodule bacteria (Rh-1, Rh-2, and Rh-5) formed convex, translucent, and slimy, rounded with smooth edges colonies with a diameter of 4 – 7 mm of a light-beige color. The cells of nodule bacteria were gram-negative, 0.5 – 1.2 μm in diameter, motile, rod-shaped, and aerobic.

Biochemical tests on nine strains showed the absence of amylase activity in bacterial strains; gelatin was liquefied by Az-13 and Rh-5 strains. The catalase activity was observed in all strains; high activity was observed in Az-34, Az-3, and Az-6 strains. The oxidase activity was observed in Az-34, Az-6, and Az-13 strains. When growing microorganisms on a medium with ferrous ammonium citrate, the formation of iron sulfide (FeS) was found in one strain (Rh-1). None of the studied strains produced ammonia. The studied strains assimilated carbohydrates and alcohols. The optimal conditions for growing strains are the following: the temperature within 25 – 28 °C and the medium acidity within pH 7.0 – 7.4 **(Table 1)**.

Table 1. Physiological and biochemical properties of the strains of azotobacter and nodule bacteria

The name of the strains	Relative to oxygen	Test catalase	Test an oxidase	Gelatin Liquefaction	Hydrolysis of starch	Hydrogen sulfide formation	Indole formation	Ammonia formation	Growth on the medium Ashby	Utilization			
										sucrose	lactose	galactose	mannose
1	2	3	4	5	6	7	8	9	10	11	12	13	14
Az-34	A	+	+	-	-	-	-	-	+	-	+ O_2	-	-
Az-6	A	+	+	-	-	-	-	-	+	+ O_2	-	+ O_2	+ O_2
Az-4/3	A	+	+	-	-	-	-	-	+	-	+ O_2	-	+
Az-9/2	A	+	-	-	-	-	-	-	+	±	+ O_2	±	±
Az-2/1	A	+	+	-	-	-	-	-	+	-	+ O_2	-	+
Az-13	A	+	+	-	-	-	-	-	+	+ O_2	+ O_2	+ O_2	+ O_2
Rh-1	A	+	+	-	-	-	-	-	+	+	±	±	±
Rh-5	A	+	+	+	-	+	-	-	+	+	±	±	±
Rh-2	A	+	-	+	-	+	-	-	+	+	±	±	±

Notice: "A" aerobe; "+" positive reaction; "-" negative reaction; " ± " weakly expressed by the positive response; "+ O_2" positive response forming oxygen.

Genetic identification of the studied bacterial strains based on the analysis of the nucleotide sequence of the 16S rRNA gene

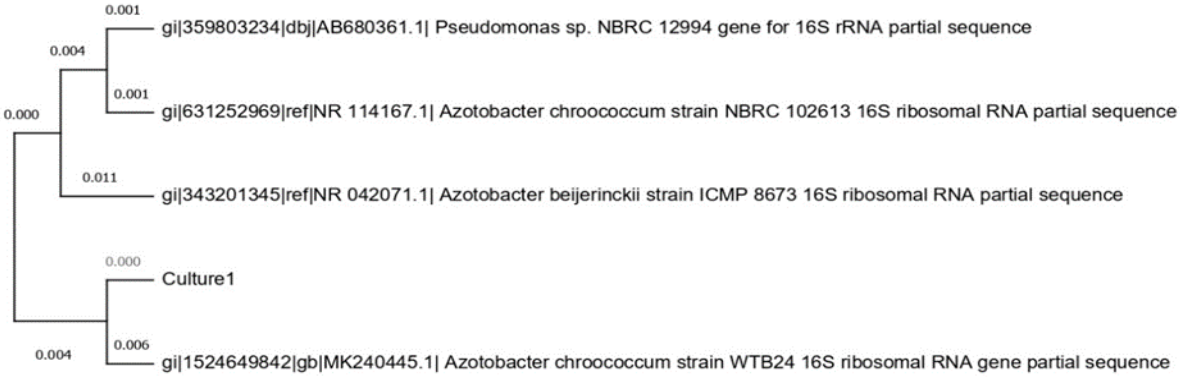
The nucleotide sequences of the *16S rRNA* gene of the identified strain were analyzed and combined into a common sequence in the SeqMan software (Applied Biosystems). After that, the terminal fragments (nucleotide sequences of primers, fragments with low-quality indices) were removed, which allowed obtaining a nucleotide sequence with a length of more than 650 bps, which was identified in GeneBank using the BLAST algorithm. The results of the genetic identification of the studied strains are shown in **Table 2**.

Table 2. The results of identification using the method of analyzing the nucleotide sequence of the *16S rRNA* gene using BLAST http://blast.ncbi.nlm.nih.gov/

No.	Strain names	Generic affiliation	% of identity
1	Az-13	*Azotobacter chroococcum*	99.80%
2	Rh-1	*Rhizobium radiobacter*	99.40%
3	Az-4/3	*Azotobacter chroococcum*	100.0%
4	Az-6	*Beijerinckia fluminensis*	100.00%
5	Az-9/2	*Azotobacter chroococcum*	97.10%
6	Az-2/1	*Achromobacter marplatensis*	100.00%
7	Az-34	*Agrobacterium tumefaciens*	98.53%
8	Rh-2	*Rhizobium legmunasarium.*	98.10%
9	Rh-5	*Agrobacterium sp.*	99.29%

The evolutionary relationships of taxa

The evolutionary history was derived using the Neighbor-Joining method [18]. An optimal tree with the sum of the branch length = 0.06861339 was shown (next to branches). The evolutionary distances were calculated using the maximum composite likelihood method [19] and expressed in terms of the number of base substitutions on site. This analysis included 13 nucleotide sequences. All ambiguous positions were removed for each pair of sequences (the pairwise deletion option). There was a total of 1,483 positions in the final dataset. Evolutionary analysis was performed in MEGA X [20] **(Figure 1)**.



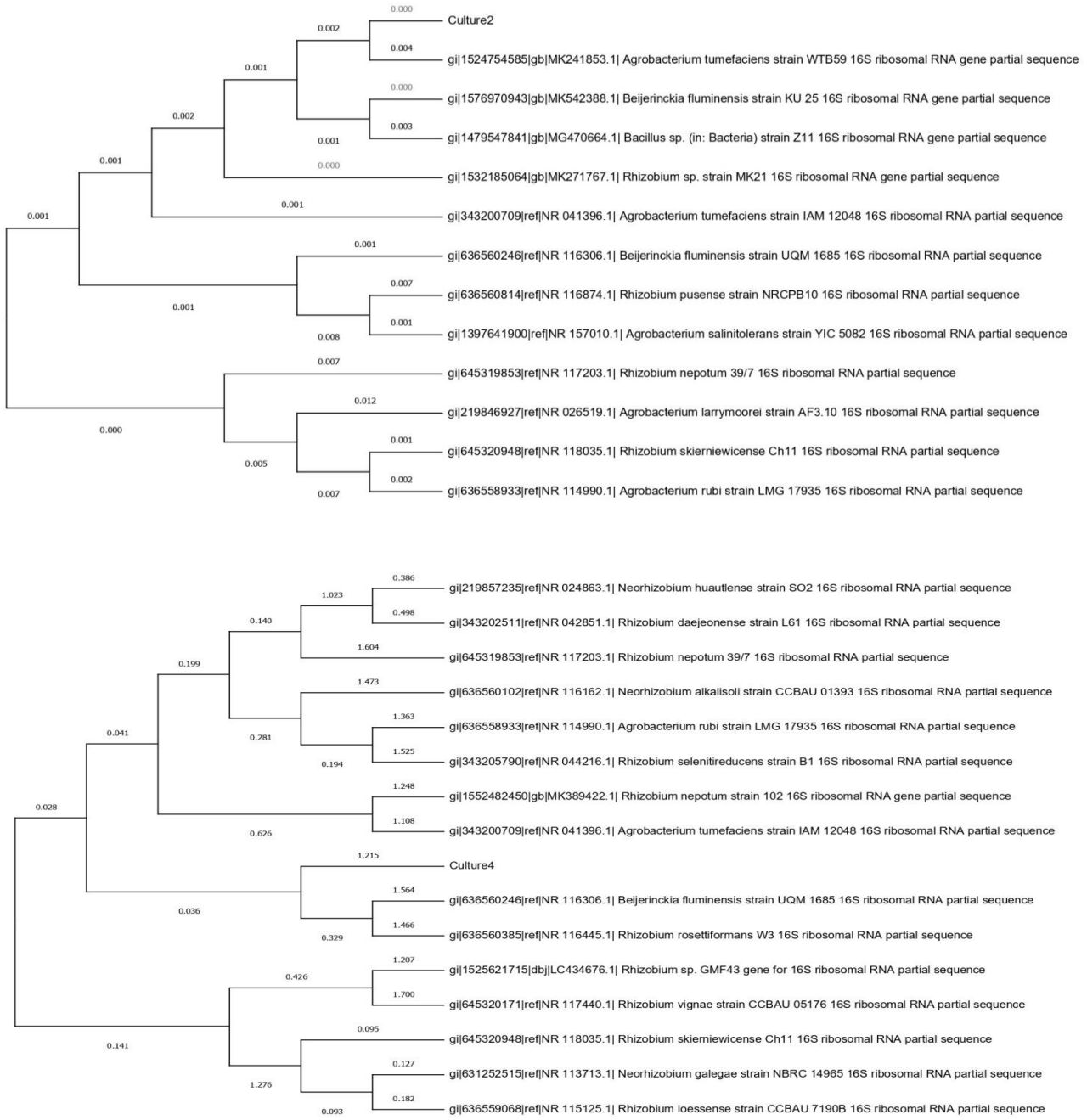


Figure 1. The phylogenetic tree of isolated bacterial strains from the rhizosphere of wheat and pea nodules

Studying the biological activity of the strains of nitrogen-fixing bacteria

The assay of the nitrogen-fixing ability of the bacteria was performed using the method of screening the studied strains grown on a nitrogen-free mineral medium (NFMM) using glucose as a carbon source **(Figure 2)**.

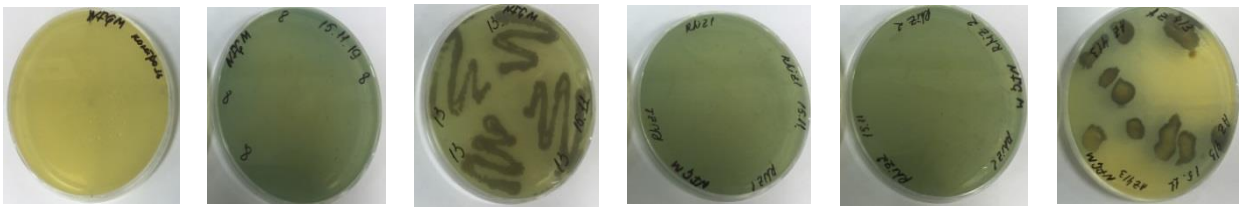


Figure 2. The assay of the nitrogen-fixing ability of bacterial strains on NFMM

The nitrogen-fixing activity was visually detected using NFMM with 0.7% glucose. Bromothymol blue (BTB) was added to the medium as an indicator. After seven days of incubation, a medium color change was noted.

The nitrogen-fixing ability of the bacterial strains was established by the yellow-green coloration of the medium, which showed the pH changes in the alkaline range; these pH changes were associated with the accumulation of ammonia ions [21].

The nitrogen-fixing ability of the bacterial strains was measured based on the measurement of the concentration of NH^+_4 free ammonium ions, which showed that the amount of fixed nitrogen in the culture medium varied from 4.5 to 41.027 μmol/l of NH^+_4 **(Table 3)**.

Table 3. Measuring ammonia accumulation by Azotobacter sp. and Rhizobium sp. strains

No.	Strain name	Empty test tube weight	Gross wet weight	Net wet weight	OD_{540}	conc. of NH_4^+ μmol/l
1	2	3	4	5	6	7
1	Rh-1	0.995	1.1787	0.1787	0.569	41.0
2	Rh-4	0.991	1.1043	0.1133	0.831	14.9
3	Az-34	0.996	1.0515	0.0603	0.284	5.1
4	Az-4/3	0.987	1.0415	0.0545	0.273	4.9
5	Az-6	0.991	1.029	0.0380	0.454	8.2
6	Rh-5	0.989	1.1139	0.1249	0.086	1.6
7	Az-9/2	0.946	1.0321	0.0861	0.058	1.0
8	Az-13	0.933	1.0291	0.0889	0.064	1.0
9	Az-2/1	0.946	1.0232	0.0772	0.023	4.5

By the ability to accumulate nitrogen, the most active were *Rhizobium leguminosarum* Rh-1, Rh-4, and *Azotobacter chroococcum* Az-34, and Az-4/3.

Studying the antifungal activity of the strains of nitrogen-fixing bacteria

A study of the antifungal activity of azotobacter and nodule bacteria cultures showed that all strains suppressed and inhibited the growth of fungal mycelium. This allows using these strains in fighting the pathogens of crops **(Figure 3)**.

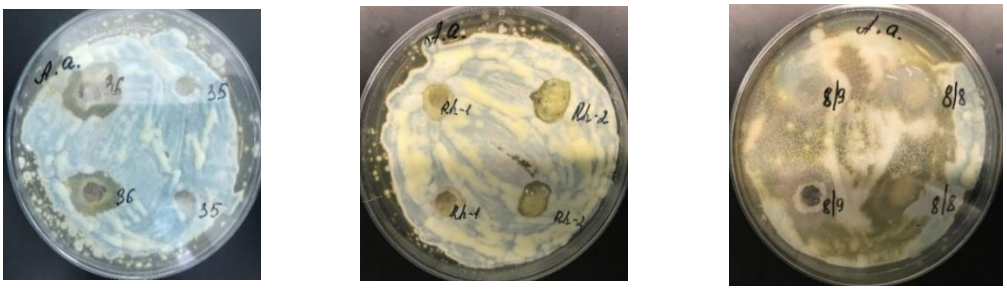


Figure 3. The zones of the *A. alternate* test fungus growth inhibition with the tested cultures (Czapek-3 agar medium (perforation method))

Studying the strains' ability to stimulate the growth of plants

In the laboratory and vegetation experiments where radish seeds were treated with Az-12, Az-8/9, Az-29, Az-24, Az-23, Az-6, Rh-2, Rh-3, Az-36, Az-28, Az-13, Az-25, and Az-22 bacterial strains, an increase in seed germination rate up to 85 – 100% was observed, while in the reference, the germination rate was only 35%. 100% germination and stimulation of root and seedling growth were ensured by Az-4, Az-28, Rh-1, Az-6, and Rh-3 bacterial strains **(Figure 4)**.

Figure 4. Stimulation of germination and growth of the seedlings of Dungansky radish seeds by the cultural liquid of microorganisms

Based on the biological properties of the bacterial cultures, three biocompatible associations of bacteria were selected from the most active strains:

association 1 – *Rhizobium legmunasarium* Rh-2, *Beijerinckia fluminensis* Az-6, *Azotobacter chroococcum* Az-9/2, and *Azotobacter spp* Az-33;

association 2 – *Rhizobium radiobacter* Rh-1 + *Agrobacterium tumefaciens* strain Az-34 + *Azotobacter chroococcum* strain Az-13 + *Azotobacter chroococcum* strain Az-4/3, and

association 3 – *Rhizobium leguminosarum* strain Rh-1 + *Rhizobium leguminosarum* strain 4 + *Rhizobium leguminosarum* strain Rh-5.

Assessment of the bioproducts effectiveness in the growing experiment

The selected association of bacteria was tested in the conditions of a greenhouse by treating the seeds of the Aina and Omskaya-36 spring wheat varieties. **Table 4** shows the data about the structure of spring wheat yield.

Table 4. The yield structure of the Aina spring wheat variety upon the use of various products in the vegetation experiment

Strain association	Stem length, cm	Spike length, cm	Stem weight, g	Spike weight, g	Number of grains per spikelet, pcs	Total grain weight, g	Single grain weight, g
Reference	42.0	13.0	0.98	1.64	24.0	0.48	0.02
Association 1	47.2	9.6	0.91	0.716	16.0	0.416	0.026
Association 2	59.0	13.5	1.45	1.04	34.0	0.952	0.028
Association 3	46.6	9.6	0.96	0.73	18.0	0.468	0.026

The results of the structural analysis showed that the plant height was the best in association No. 2 – 59.0 cm, while in the reference, it was 17 cm lower. The stem length was the smallest in the variant with association No. 3. The best conditions for weight accumulation in a single grain of a spike were formed with variant No. 2 and amounted to 34 pcs.

The grain quality in terms of nitrogen and protein in association No. 1 was 1.38% and 7.87% higher than in the reference, and in association No. 3 – 1.08% and 6.16% higher, respectively. The best indicators were observed in the variant with association No. 2 **(Figure 5)**.

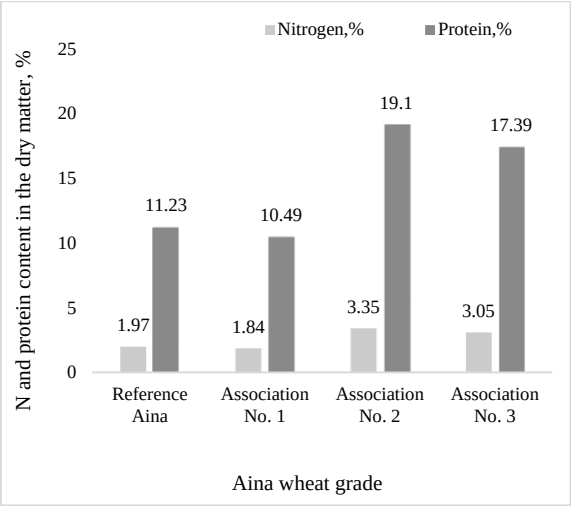


Figure 5. Accumulation of nitrogen and protein in spring wheat seeds after treatment with various bacterial associations

The content of nitrogen and protein in the dry matter of the Omskaya-36 spring wheat variety also exceeded that in the reference. The difference between the reference sample and the samples treated with the strains of association No. 3 was 0.87% and 4.96%, with the strains of association No. 1 – 0.77% and 4.39%, with the strains of association No. 2 – 0.35% and 2%, respectively.

It was found that the highest nitrogen and protein content had been achieved upon treatment with *Beijerinckia fluminensis* Az-6 strain in the dry matter with the nitrogen content of 3.15% and with the protein content of 19.69%, Az-28 - N strain in the dry matter with the nitrogen content of 3.28% and the protein content of 20.5%, Az-3 - N strain in the dry matter with the nitrogen content of 2.71% and the protein content of 16.64%, the reference - N in the dry matter with the nitrogen content of 2.77% and the protein content of 17.31%, Rh-3 strain in the dry matter with the nitrogen content of 2.98% and the protein content of 18.63%, *Agrobacterium sp.* Rh-5 - N in the dry matter with the nitrogen content of 3.08% and the protein content of 19.25%, and the reference - N in the dry matter with the nitrogen content of 2.77% and the protein content of 17.31%. The content of nitrogen and protein in the studied associations was shown. For association No. 1 – N in the dry matter, the content of nitrogen was 2.81% and the content of protein was 17.56%, for association No. 2 – N in the dry matter, the content of nitrogen was 2.59% and the content of protein was 16.19%, for association No. 3 – N in the dry matter, the content of nitrogen was 2.84% and the content of protein was 19.25%. and for the reference – N in the dry matter, the content of nitrogen was 2.77% and the content of protein was 17.31%.

The biological product effectiveness was assessed in a field experiment in the experimental fields of LLP Agricultural Experimental Station Zarechnoye

Based on the results of the vegetation experiment, the second association was selected for testing in the experimental field of LLP Agricultural Experimental Station Zarechnoye: *Rhizobium radiobacter* Rh-1 + *Agrobacterium tumefaciens* strain Az-34 + *Azotobacter chroococcum* strain Az-13 + *Azotobacter chroococcum* strain Az-4/3. For this purpose, seeds of the Omskaya-36 spring wheat variety had been treated with a suspension of association No. 2 with the titer of bacteria of 10^9 CFU/ml. The reference was the variant without wheat seeds treatment with the product. The experiment scheme was the following:

Variant 1 – reference (wheat seeds without treatment),

Variant 2 – the biological product,

Variant 3 – Lamador chemical product, and

Variant 4 – Lamador chemical product + the biological product.

The total plot area was 1,440 m^2 (0.144 ha), the area of each plot was 160 m^2, the distance between the rows was 2 m, and the length was 80 m.

The consumption of the biological product was 450 ml of the suspension of the biological product per plot; a 40% sucrose solution was used as an adhesive. The experiments were repeated four times.

The results of assessing biometric indicators of spring wheat are shown in **Table 5**.

Table 5. Biometric indicators of wheat at the end of the vegetation season

Variant	Plant height, cm	Productivity, pcs	Spike length, cm	Number of grains per spike, pcs	Grain weight in 25 plants, g	Weight of 1,000 grains, g

Reference	48.5 ± 0.08	1.26 ± 0.03	8.2 ± 0.06	20.37 ± 0.39	22.47 ± 5.00	34.9 ± 1.22
The biological product	45.7 ± 0.37	1.62 ± 0.03	8.8 ± 0.07	21.25 ± 0.13	32.97 ± 0.58	40.5 ± 0.35
Lamador reference	48.4 ± 0.56	1.25 ± 0.02	8.82 ± 0.01	20.45 ± 0.35	25.2 ± 0.65	39.7 ± 0.41
The biological product + Lamador	48.2 ± 0.17	1.79 ± 0.04	9.2 ± 0.07	22.55 ± 0.49	38.15 ± 1.32	39.05 ± 0.41

The data in **Table 5** show that the biological product had a positive effect on wheat productivity and grain weight, compared to the reference. However, the effectiveness of the product was higher upon its use with the Lamador seed dresser. For instance, the weight of grains from 25 plants in the variant with the biological product was 32.9 g, and in the variant with Lamador + the biological product – 38.15 g, respectively, and in the reference variant – 22.47 g. The spike length was greater in the Lamador + the biological product variant; it was smaller in the variant with the biological product, and it was the smallest in the reference variant.

Table 6 shows the data about grain quality by the variant of the experiment

Table 6. Test results as to grain quality indicators

Variant of the experiment	Protein,%	Humidity,%	Gluten,%	GDM,%
Reference without treatment	14.40 ± 0.11	11.82 ± 0.17	25.12 ± 0.34	83.75 ± 0.4
Lamador reference	14.47 ± 0.03	13.17 ± 0.19	25.70 ± 0.22	83.00 ± 0.65
The biological product	14.60 ± 0.08	13.60 ± 0.13	26.45 ± 0.33	82.25 ± 0.13
The biological product + Lamador	15.35 ± 0.06	15.97 ± 0.27	27.35 ± 0.09	84.25 ± 0.45

The data in the **Table 6** show that the grain quality of the Omskaya-36 spring wheat variety in terms of protein and gluten content was higher in the variant with the biological product + fungicide treatment. With the humidity of 15.97%, the mass fraction of crude gluten was 27.35%, the quality of crude gluten GDM (Gluten Deformation Meter) was 84.25%, and the mass fraction of protein was 15.35%. The values in the variants with the biological product were not much higher than those in the reference variant of the experiment.

The weather conditions of the 2019 vegetation season in the Kostanay region were extremely unfavorable for wheat. Cold weather during the germination period and excessive drought during the vegetation season prevented the biological product to show its effectiveness in full. Nevertheless, the Lamador + biological product variant provided a 14.1% increase in the grain yield **(Table 7)**.

Table 7. Assessment of the biological product effectiveness with the Omskaya-36 spring wheat variety

Variant	Threshing yield, kg	Mowing area, m^2	Obtained yield, 100 kg/ha	Humidity, %	Yield rate at 14% humidity
Reference	3.17 ± 0.11	35.75 ± 0.20	8.85 ± 0.32	10.57 ± 0.37	9.22 ± 0.30
The biological product	3.22 ± 0.09	35.72 ± 0.22	9.02 ± 0.23	14.82 ± 0.388	8.95 ± 0.21
Lamador reference	3.35 ± 0.08	35.95 ± 0.24	9.3 ± 0.17	14.77 ± 0.21	9.25 ± 0.18
Lamador + the biological product	4.17 ± 0.11	35.97 ± 0.301	11.6 ± 0.26	22.02 ± 0.41	10.52 ± 0.25
LSD_{05}	-	-	1.20	-	-

In the reference, the wheat grain yield was 885 kg/ha. In the best variant of the experiment (Lamador + the biological product), the yield was 1,160 kg/ha. The positive increase in the yield was 14%.

Thus, despite the unfavorable weather conditions, the biological product had a positive effect on the yield and quality of the wheat grain, but its effect was higher on the background of the seed dresser. This allows recommending the product for use in the existing technological system of wheat cultivation.

To increase the yield rate of crops in the Kostanay region, the nitrogen-fixing bacteria-based biological product was developed. Out of nine promising strains, four strains of microorganisms were selected that met the criteria of the growth-stimulating bacteria, nitrogen-fixing bacteria, and antagonist bacteria to phytopathogenic microorganisms. Vegetation and field studies of the effectiveness of liquid forms of the products based on bacterial associations for wheat crops were performed. The results of the laboratory and vegetation experiment showed that the quality of spring wheat grain treated with biological products in terms of total nitrogen and protein was higher, compared to the reference variant, therefore, bacteria association No. 2 was chosen as the best variant.

The weather conditions of the 2019 vegetation season were extremely unfavorable for wheat. Acute drought in the critical phases of growth resulted in the fact that the biological product could not show its effectiveness in full; however, the variant where the seeds were treated with the biological product and the dresser provided a 275 kg/ha increase in the wheat grain yield. The effectiveness of the product is to be proven in more favorable weather conditions.

The authors' results as to the effectiveness of associations of nitrogen-fixing bacteria have several matches with the studies of other scientists. The synergistic relationship of azotobacter with the interaction of rhizobium as coinoculants was observed in

most studies performed in laboratory, greenhouse, or field conditions. For instance, Siddiqi *et al*. reported that *Azotobacter chroococcum* (AC.C-112) together with the *Rhizobium* (RS.C-112) strain significantly increased the nodulation, dry weight, and grain yield (by 49.2%) compared to the reference, which indicated a positive effect of the *Azotobacter* and *Rhizobium* (AC.C-112 + RS.C-112) mixed inoculate. *Azotobacter chroococcum* (AC.C-112) together with the strain of the *Rhizobium* species (RS.C-112) significantly increased the nodulation, dry weight, and grain yield (by 49.2%), compared to the reference, which indicated the beneficial effect of the *Azotobacter chroococcum* and the *Rhizobium* species (AC.C-112 + RS.C-112) mixed inoculate [22].

Seed treatment with a bacterial composition of the *Rhizobium* and *Azotobacter* genera had a positive effect on the growth of the seeds. The weight of the shoots obtained from these seeds was greater than that of the seeds treated with the reference product. Upon combined inoculation with *Azotobacter* and *Rhizobium spp.,* a positive response of the crops was observed. The significant increase in the nodulation of *Azotobacter spp*. has a strong effect on the activity of *Rhizobium spp*. [23].

Conclusion

The results of the studies showed that the use of biological products of associative nitrogen-fixing microorganisms for spring wheat in the extreme weather conditions of 2019 has contributed to a 250 kg/ha increase in the yield. The biological product's compatibility with the Lamador chemical fungicide manufactured by Bayer Schering Pharma has also been established.

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