Study of the Influence of Nitrogen-fixing Biological Products on the Yield of Grain Legumes of Northern Kazakhstan Used in the Food and Agricultural Sector

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Abstract. The article presents data on the development of a biological product, based on new strains of nitrogen-fixing microorganisms of nodule bacteria of the *Rhizobium* genus, stimulating replenishment of biological nitrogen and soil nitrogen pool to increase the productivity of agricultural crops in Northern Kazakhstan. The experimental data are based on the use of obtained grain legumes, which are sources of vegetable protein and are able to solve the food problem, often help to improve the quality of products, as well as effect saving in raw materials, and give the products dietary and therapeutic targeting.

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Introduction.

The symbiosis of leguminous plants and nodule bacteria is a unique natural replenishment system of soil nitrogen. Evolution of symbiosis is accompanied by a complex chain of genetic relationships. This results in fixation of molecular nitrogen from atmosphere by rhizobia and hosting plant, allowing full development of legumes without introducing into the soil costly and environmentally harmful nitrogen compounds [1]. Interest in rhizobia arose, first, due to agricultural importance of the symbiotic nitrogen fixation, and secondly, due to the fact that the legume-rhizobium symbiosis is one of the most interesting types of plant-microbe interactions [2]. Fixation of atmospheric nitrogen by nodule bacteria of the *Rhizobium* genus in the symbiosis with leguminous plants is the subject of focused attention of scientists around the world. Study of the mechanism formation of such symbiosis, specificity factors, determining the possibility of the formation of nitrogen-fixing nodules by rhizobia strain on a particular plant, has both theoretical and especially practical interest [3].

Protein of legumen crops is rich with indispensable amino acids that are essential to animals and humans though cannot be synthesized by their body. Furthermore, legumes, when leaving plant residues in the soil, increase soil fertility [2-4]. Application of biological products in the legume crops strengthens the activity of soil nitrogen-fixers [5].

The problem of obtaining fundamentally new formulations that would both provide a comprehensive mineral nutrition of microbial cells and at the same time be environmentally friendly, is to date very urgent. Complex mineral fertilizer, based on the immobilization of microorganisms, is a fundamentally new type of created biological product.

Legumes are very important from both an environmental and agricultural viewpoints, since they are responsible for a substantial part of the global nitrogen flow from the atmosphere into the fixed form (ammonia, nitrate, and organic nitrogen).

All legumes are used successfully in medical practice. Legumes can be considered as therapeutic treatment foods [6]. The effectiveness of their use as prophylaxis of gastrointestinal tract, cardiovascular system, kidneys and liver has been already proven. Ideally, legumes should be included to our diet at least at 8-10%.

For example, green peas include the active unti-sclerotic substances. They protect the body against infections and purify the blood [7]. Beans contain large amounts of B vitamin, which reduces the risk of cardiovascular diseases; have a beneficial effect on digestion because they contain a lot of dietary fiber [8].

Based on the foregoing, we tasked to develop a biological product, based on nodule bacteria, to be used for dropping legumes. To this end, we set a goal to generate new strains of nitrogen-fixing microorganisms, since the efficiency of a designed biological product is primarily determined by the activity of nodule bacteria strain, which contributes to the compensation of biological nitrogen and soil nitrogen pool, improves soil fertility and crop yields in Northern Kazakhstan [9].

Material and methods

Recovery of pure nodule bacterial culture. Recovery of nodule bacteria from soil was performed using the microvegetation method. Soil samples were selected from experimental fields of Shortandy and "Dostyk-06" LLP. Each sample was taken in an amount of 1 gram after thorough mixing of the soil. The samples were suspended in 2 ml of distilled water and incubated overnight by means of shaking (at a temperature of 28^0C and rotation rate 180 rpm). Then the solutions were centrifuged for 3 minutes at 3000 rpm. The supernatant fluid was used as a mixture for inoculation of pea seeds (*Pisum sativum L*) of Omsk non-shatter breed, chickpea (*Cicer arietinum L*) of Krasnokutsk breed, and lentils (*Carpodacus erythrinus*) of Wehowski greed. Sterile two-day seedlings of host plant were inoculated with soil suspension, prepared in an amount of 1 g of soil per 10 ml of water. The plants were grown for 30 days in a sterile soil placed in 5-liter vessels. Largest pink nodules were separated from the plant's root by means of tweezers, than washed thoroughly in tap water. The nodules were placed in a porcelain cup with 96% ethyl alcohol for several minutes. Thereafter nodules were repeatedly washed with sterile water and transferred by means of sterile tweezers to a sterile Petri dish and cut into pieces by a sterile knife. A small amount of nodule content was transferred by means of inoculation loop into a drop of sterile water situated on the surface of the agar medium in a Petri dish, and smeared with a spreader. The same spreader was used to make sowings on another 2-3 plates to obtain single colonies (exhaustive inoculation). Inoculated cups were incubated in thermostat at 25-27°C.

Study of morphological and biochemical properties of nodule bacteria was performed by conventional techniques [10-11].

Micro-vegetation experiments. Micro-vegetation experiments were conducted in the 40 liters vessels. The experiments were carried out in four replications. Pea seed in an amount of 49 pieces, treated by bacteria culture fluid with a titer of 10^8, were placed in each vessel. Seed germination was determined by the number of seedlings planted respective to the total amount of seeds.

Preparation of inoculant. Ten ml of culture fluid of nodule bacteria with a cells titer 10^9 cells/ml were mixed with sterile carrier in an amount necessary to achieve 40% moisture. Inoculants were stored at 25^0C in the 250 ml flasks. Each inoculant was prepared in three replications. Cell viability was calculated by sowing of 10-fold dilutions on pea agar.

Field trials of experimental forms of nitrogen-fixing biological products. Experiments were conducted on test areas of "Baraev Grain Hasbandry SPC" LLP in three replications. Leguminous crops with seeds that were not treated by nodule bacteria biomass, as well as pea seed crops, treated with rizotorfin, which was produced by Production Research Institute for Agricultural Microbiology in St. Petersburg, served the control data. The area of each plot was 100 m^2 (25 x 4 m).

Soil sampling was carried out by an envelope technique on the depth of the plow layer (0-10, 10-20, and 20-30 cm).

DNA purification from the test strains. Purification of genomic DNA was performed out of nocturnal culture using a set of «Genomic DNA purification Kit» (Fermentas, EU). To amplify a fragment of 16S r DNA by PCR method, the following primers were used: forward primer f – AgAgTTTgATCCTggCTCAg; and reverse primer r – ggACTACCAgggTATCTAAT.

Electrophoresis was performed in 1.0% agarose gel, followed by extraction of DNA fragments (corresponding to 800 bp) by means of «DNA Extraction Kit» (Fermentas, EU).

Electrophoretic separation of the amplified fragments. To observe the selected DNA fragments, the electrophoreses was carried out at a voltage of 5 V/cm^3 over 1 hour. For this purpose, 1% agarose was prepared. Single-use TAE solution (40 mM of Tricine, 40 mM of SN3SOOH, and 2 mM of EDTA-Na pH-8.0) was used as an electrophoretic buffer. One gram of agarose per 100 ml of distilled water was heated until complete dissolution, followed by adding 5 mcL of etidium bromide (10 mg/ml). Then slightly chilled agarose was poured into plates with a comb to cool down. Next a dilute solution of 10x TAE was poured on top of stiffen gel. Further, 5 mcL of the test sample, stained with «loading buffer», was introduced into the agarose seedbeds.

Carrying out amplification of 16s RNA by PCR. A reaction mixture in amount of 30 mcL was prepared. The elaborated protocol of 16s RNA (3 mcL of buffer for PCR, 3 mcL of MgSO4, 3 mcL of dNTRs - a mixture of free nucleotides, 1 unit of the Tag-polymerase enzyme (5 U/mcL), specific PCR-2 primers (forward primer 8 - AGA GTT TGA TCC TGG CTC AG, and reverse primer 806 - GGA CTA CCA GGG TAT CTA AT), 2 mcL of the test sample, deionized water was taken in amount of 16.5 mcL per each reaction mixture) was used for amplification.

Amplification mode (96ºC – 4 min, 96ºC – 10 sec, 55ºC – 5 sec, 60ºC – 4 min, and 20ºC – ∞).

Product PCR purification. Common reaction mixture in total volume of 10 ml was prepared for all the samples.

The protocol for PCR product purification: (SAP – 0.5 mcL, EXO – 0.25 mcL, buffer - 1 mcL,

$MgSO_4$ – 2.5 mcL, and H_2O – 5.75 mcL).

A protocol for sequencing PCR was drawn up after PCR products purification by means of two digestive enzymes.

Mode for the PCR product after purification: (37ºC – 30 min, 85ºC – 10 min, and 4ºC - ∞). For sequencing PCR, the total reaction mixture was prepared separately for each primer. The final amount of the reaction mixture was 15 mcL.

Protocol for the forward primer: (Big dye – 1 mcL, buffer – 1 mcL. Forward primer for PCR – 0.4 (3,2 pmol) – 6 mcL, and H_2O – 5.6 mcL).

Protocol for the reverse primer: (Big dye – 1 mcL, buffer – 1 mcL. Reverse primer for PCR – 0.4 (3.2) – 2 mcL , and H_2O – 5.6 mcL).

PCR sequencing mode: (96ºC – 4 min, 96ºC – 10 sec, 55ºC – 5 sec, 60ºC – 4 min, and 20ºC - ∞).

Purification of sequencing product. The protocol for the purification is given below; its total volume amounts 30 mcL for each sample (AcNa (sodium acetate) – 6 mcL, H_2O (deionized water) – 6 mcL, and 96% ethanol – 138 mcL).

Exploratory studies of biological product. Exploratory studies of biological product, based on nitrogen-fixing bacteria, was carried out in «Electrolux» fermentors (Sweden) of 7 and 130 liters at the subsidiary of «National Center for Biotechnology of the Respublic of Kazakhstan" in Stepnogorsk.

Results and discussion.

We have employed micro-vegetation technique to recover 12 isolates of nodule bacteria sampled from soils under legumes, growing in the Akmola region. It is known that some strains of nodule bacteria are capable of cross-infection, i.e., capable to induce the formation of nodules on the roots of leguminous plants of other species.

In this regard, except of bacteria, recovered from pea nodules *(Pisum sativum L)* of the Omsk non-shatter breed, in our research we have used bacteria, recovered from nodules of chickpea plants *(Cicer arietinum L.)* of Krasnokutsk 23 breed, as well as lentils *(Carpodacus erythrinus)* of Wehowski breed. Six isolates, most similar to rhizobia, were selected based on morphological characteristics. When micro-copying, they represented single spaced, short, gram-negative rods with rounded ends. Figure 1 shows the morphology of culture cells, recovered from nodules of *Pisum sativum L.* of the Omsk non-shatter breed, strains A1 and A2. We have used also bacteria, recovered from nodules of chickpea plants *(Cicer arietinum L.)* of Krasnokutsk 23 breed, strains B1 and B2, as well as lentils *(Carpodacus erythrinus)* of Wehowski breed, strains C1 and C2.

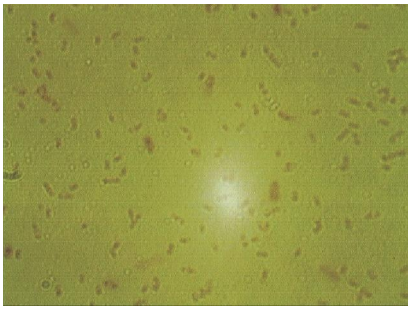


Figure 1. Morphology of the microorganism cells, isolated from nodules of leguminous plants (a - *Carpodacus erythrinus*)

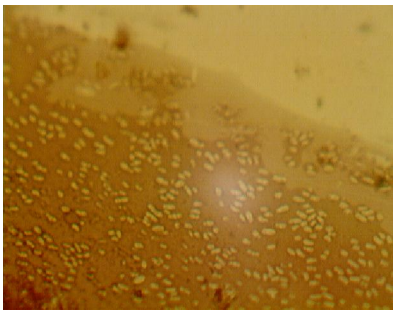


Figure 1. Morphology of the microorganism cells, isolated from nodules of leguminous plants (b - *Cicer arietinum L.*)

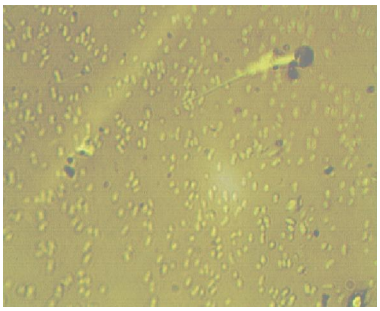


Figure 1. Morphology of the microorganism cells, isolated from nodules of leguminous plants (c - *Pisum sativum*)

The test cultures are non-acid-resistant and do not form spores. Recovered microorganisms are mobile, having cell width of 0.5-0.9 microns and the length of 1.2-3.0 microns.

We have studied the physiological and biochemical properties of selected micro-organism cultures. At that, we determined the milk acidification, the formation of H_2S, NH_3, and the use of various carbohydrates.

All studied bacteria cultures are aerobic microorganisms, forming the transparent mucous zone on a milk surface. Development of bacteria in the milk with litmus did not alter the environment, though slightly alkalized it. Only a culture of bacteria

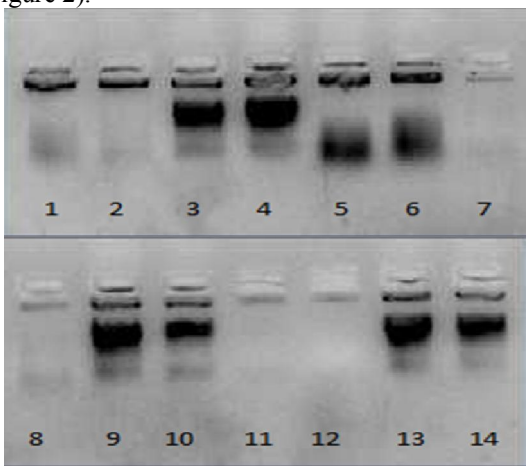
B1 and B2 acidified milk, bringing it to a bright pink color. All bacterial cultures grow well on the Ashby medium, they are catalase-positive, not hydrolyzing starch.

Variability of isolates symptoms was observed when growing on carbohydrate-containing environments. Most cultures well assimilate arabinose, forming an alkaline medium. At that, it is noted that while growing they have little use of mannitol and glycerol.

The above mentioned set of phenotypic characters was insufficient to establish the taxonomic status of the tested bacteria. Therefore, we performed a genetic identification of bacteria that are widely used by 16,000 gene sequences 16s RNA, which are presented in the available database [12].

Identification of nodule bacteria is based on the detection and amplification of gene fragment by polymerase chain reaction, which is three frequent cyclically repeating processes: thermal denaturation of DNA in the test sample; the hybridization (annealing) of the target DNA with specific oligonucleotide probes (primers); synthesis of complementary DNA chains using a thermally stable DNA-polymerase, isolation of DNA aggregation, the PCR, i.e. amplification reaction of a 16s RNA gene fragment, and the electrophoresis of PCR products in an agarose gel.

To purify the DNA from tested microorganisms, we took 12-hours cultures, grown on solid culture medium in a thermostat at 30ºC. Purification of DNA from selected antagonist strains was carried out using a DNA isolation kit «Wizard Genomic DNA Purification Kit» produced in the USA by «Promega corporation». After purification of DNA, we performed electrophoretic analysis of the samples (Figure 2).

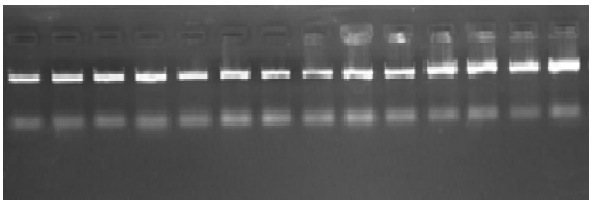


Control DNA (1, 2); A1 (3, 4); A2 (5, 6); B1 (7, 8); B2 (9, 10); C1 (11, 12); and C2 (13, 14).

Figure 2. Electrophoretic separation of DNA after amplification.

In Figure 1 one can observe DNA bands in double replications, as well as RNA bands, because we did not split ribonucleic acids.

Further, we performed the amplification of 16S rDNA fragments of the studied strains (about 800 bp). Strains were amplified using forward primer (8 - AGA GTT TGA TCC TGG CTC AG) and reverse primer (806 - GGA CTA CCA GGG TAT CTA AT). Amplification was performed using the Bio Rad 2 amplificator. DNA concentration and purity was assessed by electrophoresis in comparison with the λHind III marker, having DNA fragments of a known concentration (Figure 3).



Control DNA (1, 2); A1 (3, 4); A2 (5, 6); B1 (7, 8); B2 (9, 10); C1 (11, 12); and C2 (13, 14).

Figure 3. Electrophoretic separation of the DNA after amplification.

After electrophoresis, a purification of PCR products was performed using two enzymes, one of which (SAP - phosphatase) cleaves foreign nucleotide residue from the bottom and the top, in addition to the main strip. Second enzyme (EXO-exonuclease) purifies the main strip from both sides. After purification of the PCR products by means of two digestive enzymes, the protocol for sequencing PCR was drawn up.

For sequencing of PCR, the total reaction mixture was prepared separately for each primer. The final amount of the reaction mixture should be 15 mcL. Sequencing of the PCR product was carried out on *Tet Rad 2* amplifier produced in the USA. After sequencing the purification of the product was performed by sodium acetate. Sequencing of the pure PCR products were carried out on 3730hL DNA Analyzer. Sequencing allowed us to determine about 800-1000 pairs of 16S rRNA gene nucleotides of the studied strains. Next, using the GenBank database we searched for sequences, homologous to sequenced 16S rDNA sequence [13-14].

Table 1 presents data on the genetic sequence of the investigated samples and their percentage fidelity. PCR products 16S rRNA from each isolate were sequenced, and nucleotide sequences of all strains investigated were compared to NP 16S rRNA sequences, available in the international database (Table 1). This comparison has shown that two strains were 92% and 100% identic to *Rhizobium*

leguminosarum, two strains were 98% identic to the *Rhizobium sp* genus, and 99% were identic to the *Rhizobium cicer sp.* genus. Other cultures were referred to *Agrobacterium sp., Enterobacter sp.*

Table 1. Identification of microorganisms recovered from nodules of leguminous crops.

№	Strain	Fragment structure	Homo-logy %	Identified type
1	2	3	4	5
1	A1	CGAGCGCCCCGCAAGGGGAGC GGCAGACGGGTGAGTAACGCG TGGGAATCTACCCTTGACTAC GGAATAACGCAGGGAAACTTG TGCTAATACCGTATGTGTCCTT CGGGAGAAAGATTTATCGGTC AAGGATGAGCCCGCGTTGGAT TAGCTAGTTGGTGGGGTAAAG GCCTACCA	100%	*Rhizobium leguminosarum*
2	A2	TCGAGCGCCCCCGCAAGGGGA AGCGGCAGAACGGGTGAGTA ACGCGTGGGAAATCTACCCTT GACTACGGAATAACGCAGGGA AACTTGTGCTAATACCGTATG TGTCCTTCGGGAGAAAAGANT TTATCGGTCAAGGATGAGCCC GCGTTGGATTAGCTAGTTGGT GGGGTAAAGGCCTACCAAGGC GACGANTCCATAGCTGGTCTG AGAGGATGATCAGCCACATTT GGGACTGAGACACGGCCCAAA CTCCTACGGGAGG	99%	*Rhizobium cicer*
3	B1	TTTCGAGCGCCCCGCAAGGGG AGCGGCAGACGGGTGAGTAAC GCGTGGGAATCTACCCTTGAC TACGGAATAACGCAGGGAAAC TTGTGCTAATACCGTATGTGTC CTTCGGGAGAAAGATTTATCG GTCAAGGATGAGCCCGCGTTG GATTAGCTAGTTGGTGGGGTA AAGGCCT	98%	*Rhizobium sp.*
4	B2	GCATGCCACGACAATCGCTGT CCTTACAAACTAGCTGCCCGA CGCGATGCACACGCGGCCGCA TGCCGCGCAGTCCCTATTTCTT CGTCCGAACAGGGCGGCAGAT TGCTGCATCAATACGTGCACC GGGTGTTCTGGCGACTATCCC GGCAAATTGGTTTACGGCAGG AGGGGGATCGCGGTGGCCCGC GGGTTCACGATCTTCGTCACC GTTTCGCCGTCCAAAC	98%	*Agrobacterium sp.*
5	C1	CGAGAGCTTGCTCTCGGGTGA CGAGTGGCGGACGGGTGAGTA ATGTCTGGGAAACTGCCTGAT GGAGGGGGATAACTACTGGAA ACGGTAGCTAATACCGCATAA CGTCGCAAGACCAAAGAGGG GGACCTTCGGGCCTCTTGCCA TCAGATGTGCCCAGATGGGAT TAGCTAGTAGGTGGGGTAACG GCTCACCTAGGCGACG	99%	*Enterobacter sp.*
6	C2	GCCTTCGCCTTTTTTCTGTTCC TCCGAATATCTACGAATTTCA CCTCTACACTCGGAATTCCACT CACCTCTTCCATACTCCAGATC GACAGTATCAAAGGCAGTTCC AGGGTTGAGCCCTGGGATTTC ACCCCTGACTGATCGATCCGC CTACGTGCGCTTTACGCCCAG TAATTCCGAACAACGCTAGCC CCCTTCGTATTACCGCGGC	92%	*Rhizobium leguminosarum strain*

On the basis of conducted molecular-genetic identification methods we determined the sequences, which were absolutely homologous to sequenced ones. Thus, 4 strains out of recovered microorganisms were referred to nodule bacteria of the *Rhizobium* genus.

The drugs, such as Rizotorfin and Rizobin, have received particular popularity in Kazakhstan. Rizotorfin, which is based on peat together with an active strain of nodule bacteria depending on a certain plant culture, was quite intensively used in the south of Kazakhstan when cultivating soya crops. In Kazakhstan there are biological products, based on nodule bacteria of leguminous crops, i.e. soybeans. Nodule bacteria, adapted to the southern region of the Kazakh Republic, form a basis of noted product [15-16].

To compare the efficacy of developed inoculant preparative formulations with Russian Rizotorfin preparation, we have conducted small plot field experiments at "Baraev Grain Hasbandry SPC" LLP [17]. The area of each plot was 100 m^2. Experiments were performed in triplicate.

Accumulation of nitrogen in the green mass of plants was determined in the phase of branching, flowering and appearance of beans. Analysis findings showed that the highest content of total nitrogen in pea crops during the branching phase was observed in the plants treated with the A1-strain based inoculants and was 1.4 times higher as compared with the control, inoculated by a powdered inoculant, and 1.3 times higher than that when using the paste-like formulation. A slight excess by factor of 1.04 was observed in the nitrogen content of the plants treated by Rizotorfin, compared to the control data.

During the flowering period, all inoculated plants had increased nitrogen content as compared to that in the control plants. Thus, the average nitrogen content was 1.4, 1.7 and 1.6 times higher than the control values, when inoculated by powdered Rizotorfin, paste-like Rizotorfin, and inoculants, respectively. These data correlate with the data on the number of nodules on the roots of pea plants during branching and flowering. The greatest accumulation of nitrogen was noted at the inoculation of pea seeds by powdered inoculant.

However, the dependence between nitrogen accumulation in plants and the number of nodules on the plant roots was not observed during the beans appearance period. Thus, the maximum nitrogen content was observed in the plants, inoculated by Rizotorfin; amount of nitrogen accumulated in the green mass, compared to the control, was 16.1 mg/plant (greater by factor of 1.06), whereas when using domestic inoculants, growth in nitrogen accumulation was just 9.4 mg/plant (exceeding control values by factors of 1.01 and 1.04, respectively), when inoculating by powder and paste-like inoculants.

Perhaps this is due to the fact that all the fixed nitrogen is consumed and its accumulation takes place in other vegetative parts of the plants, such as

pea grains or roots. The content analysis of the protein in pea grains revealed that, when using inoculants based on biomass of nodule bacteria of A1 strain and Rizotorfin, the content of the protein in grain is higher as compared with the control (Table 2).

The highest protein content was determined in the case of using the powder product (exceeding the control value by 1.5%); when treating pea seeds by paste-like inoculant and Rizotorfin, protein content in grains accounted for 22.1% and 22.2%, respectively, that is by 0.6 and 0.7% higher than protein content in the control plants.

At the end of the growth season, a structural analysis of plants was carried out, for which 10 plants from each plot were selected. The results shown in Table 2 represent the average values. When studying the efficiency of pea inoculation in the field experiment, it was noted that inoculated pea crops are thicker than control plants (Table 2).

The stimulatory effect of pea seed treatment by inoculants, based on A1 strain, with regard to the indicators of structural analysis of pea plants, was much higher than that for seeds treated by Rizotorfin.

The highest yield of peas was harvested from the plots inoculated with powder inoculants. As compared with the control plots, the harvest from each square meter of inoculated plots was higher by 150 grams.

Table 2. Protein content in field pea grain and indicators of structural analysis of pea plants.

Parameters	Control	Risotorphin	Powder form of inoculate	Paste-like form of inoculate
Protein content in a grain, %/100 g	21.5	22.2	23	22.1
Structural analysis of the plants				
Number of plants, itm./m^2	76.8± 3.2	78.1± 2.1	80.1±4.9	79.2±2.9
Plant height, cm	58.7±4.0	53.8±3.3	69.3±2.5	63.3±2.7
Plant weight, g	3.2±0.5	2.9±0.6	4.5±0.1	3.5±0.2
Number of seedpods on a plant, itm.	5.8±0,2	5.7±0,4	8.1±0.3	6.1±0.4
Seedpod length, cm	27.2±0.2	28.0±0.1	36.9±0.2	33.9±0.7
Total grain weight from one plant, g	4.1±0.1	4.0±0.1	5.8±0.3	4.4±0.3
Number of grains per plant	20.3±1.3	20.3±0.8	27.9±1.7	22.9±1.1
Yield per 1 m^2, g	314.88	312.4	464.58	348.48

When inoculating with paste-like preparation, the excess of yield was 33.6 g, while when treating with Rizotorfin, pea yield was almost at the level of control data, more specifically, 0.02 g less per each square meter.

Study of the interaction between the plants and microorganisms is currently of particular relevance, as the use of expensive mineral and organic fertilizers, as well as other plant protection products, puts the need to find additional sources for nitrogen nutrition of plants. New strains of microorganisms were identified recently. They can inhibit the development of pathogenic micro flora that ultimately reduces the incidence of plants, increases their productivity, and improves the quality of crop production.

Conclusions.

Inoculation of pea seeds with domestic biological products accelerates and stimulates the growth of plants compared to those inoculated with made in Russia product. The greatest beneficial effect is observed when using a powder form of the preparation.

Twelve cultures of bacteria have been recovered by vegetation from the nodules of leguminous plants, growing in the northern regions of Kazakhstan. When identifying 16S rRNA gene, it was revealed that one strain out of 12 recovered cultures was 100% identical to *Rhizobium leguminosarum*.

Pot experiments revealed that the zeolite is the most effective filler for producing inoculant based on the active strain of nodule bacteria *Rhizobium leguminosarum A1*.

It was determined in field tests that the use of inoculant in powder form is more effective than that in paste-like form, as well as the Rizotorfin inoculants. Pea yield per sq. m was 1.3 and 1.5 times higher, respectively. Yield increase of pea, when using inoculants in the crop acreages of 60 hectares, was 24% at the "Dostyk 06" LLP, and 26% at the "Baraev Grain Hasbandry SPC" LLP.

Inoculation of pea seed by means of inoculants, based on the local strain of nodule bacteria, increases the biological activity of the soil and yield of peas.

The developed biological product is recommended for use in *Pisum sativum* field pea crops to improve crop yield and soil biological

activity. The obtained legumes crop was used to develop composite flour for production of bread with increased nutritional value.

The researchers of the lab of "Technology and Standardization" Department of the Kazakh University of Technology and Business have developed a formulation of composite flour for bread production out of legumes. Bread production technology and developed formulation were published previously [7-8].

Thus, the development of biological product, based on local nodule bacteria strains, and their use to increase the yields of grain legumes, is an urgent task. At the same time, the use of the obtained crop for further development of the bread production technology from wheat and legumes flour is a common technological system, which can solve the problem of providing the population with valuable, high-quality foodstuff.

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