The Biological Method of Increasing Seed Germination and Productivity of Grain Crops

3

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Contents

Abstract

Cereals such as wheat, barley, and oat are the main strategically important crops cultivated in Kazakhstan. At the same time, more than 23–30% of grain crops were lost in the fields in 2014–2015 due to low seed germination and various diseases. The main tasks of this research were to develop a biological method to enhance seed germination and protect root rots caused by fungal pathogens. From the rhizosphere of grain crops were isolated more than 50 isolates of cellulolytic bacteria. After the testing, two strains which showed no phytotoxicity and possessing growth-stimulating ability were selected. The mixture of strains when applied more actively promoted seed germination and increased seedling growth compared to single strain application. The increase of seed germination was up to 80–92%. Treatment with the association of the strains significantly suppressed root rot diseases caused by *Fusarium*, *Alternaria*, and *Bipolaris* in cereals. Molecular genetic characterization of PGPR strains used was shown to belong to the genera of *Bacillus* and *Cellulomonas* and species *B. cytaseus* and *C. flavigena*. Mode of action studies for seed germination by these strains was

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showed due to the synthesis of cellulase enzymes. The efficacy of these strains to enhance plant growth of grain crops was shown to be associated with the synthesis of biologically active substances such as B-group vitamins and amino acids. It was also established that the strain *B. cytaseus* VKPM B-4441 could fix molecular nitrogen from the atmosphere (62.5×10^{-5} N_2/ml/h). The tested strains could colonize the hard seed coat and then began to synthesize cellulase enzymes to partially degrade the seed coat to form microcracks. The seed coats then become less firm and allow the increase of water transport and dissolved mineral substances and nutrients to the seed embryo. We hypothesize that the enhanced seed germination and stimulated seedling growth are due to the process of "the biological scarification." Seeds treated with these strains prior to seeding significantly increased their germination up to 80–89% compared to control (61–65%). The root rot rating was decreased 7.3 times compared to control. The yield of grain increased in the range of 2.6–3.2 centner/ha compared with the control. On the basis of this association of strains, the new biological preparation "Batsirin A" was developed. When use biopreparation enhanced seed germination and seedling growth and protected against root rots caused by fungal pathogens.

Keywords
Cellulolytic bacteria · Seed germinations · Antagonistic activity · Nitrogen-fixing ability · Biosynthesis B-group vitamins

3.1 Introduction

The major food and fodder plants around the world are grain crops (wheat, barley, and oats). They are grown in almost 70 countries and provide bread and fodder for about half of the world's population (Vetrov 2017). Its production leads other crops, such as rice, maize, and potatoes. China has the largest land area devoted to grain production, followed closely by the United States, India, and the Russian Federation. Kazakhstan and Canada, ranking fifth and sixth, produce grain crops on about half the area of the top four countries (Curtis 2017).

Grain crops such as wheat, barley, and oats are the main strategically important agricultural crops cultivated in Kazakhstan. According to the Ministry of Agriculture, in 2016, the sown areas under all agricultural crops reached 21.7 million hectares, of which grain crops occupied 15.2 million hectares, including wheat grown on 12.2 million hectares (Aytuganov 2016). This demonstrates the high importance of these crops for an agro-industrial complex of Kazakhstan. At the same time, more than 23–30% of grain crops were lost in the fields of Kazakhstan in 2014–2015. This is caused by two main reasons: low seed germination and yield losses due to various diseases.

As a result of the economic crisis, many Kazakhstan farms are buying poor-quality seed grain with low germination capacity which leads to a significant decline in the yield of grain crops. At present, enhancing the germination of grain crops is an

urgent problem in Kazakhstan. The second reason for the yield decline is that there are the diseases of grain crops caused by the fungi belonging to the genera *Fusarium*, *Helminthosporium*, and *Alternaria*. In 2014–2015, due to diseases caused by these fungi, more than 30% of grain yields were lost on the fields of the Republic. Without solving the problems of enhancement in germination and protection from phytopathogenic microorganisms, it is impossible to improve the effectiveness and stability of grain production.

The use of chemical methods and products for protecting and enhancing the germination of agricultural crops has a number of negative consequences: formation of the stable phytopathogenic races, reduction in the number of beneficial microorganisms in microbiocenoses, and accumulation of toxic substances in the soil (Reddy et al. 2009; Fan et al. 2012; Lu et al. 2017; Pertot et al. 2017).

An alternative approach involves the development of biological methods using microbial preparations. These biopreparations are based on highly active strains of microorganisms (Novikova et al. 2003; Labutova 2011). In this regard, the use of cellulolytic bacteria is the most promising trend (Suo et al. 2011). Their physiological and biochemical properties, including high growth rate, unpretentiousness to the nutrition sources, and the simplicity of the cultivation, ensure high processability in the biomass production. In addition, due to their biological characteristics, such as the population stability and ability to synthesize antifungal metabolites, they are active fungal antagonists.

Previously, we have developed a bacteria-based biological method for increasing the germination of melilot (Smirnova et al. 2012, 2015, 2016). The method consists in the application of cellulolytic bacteria synthesizing cellulase enzymes that partially degrade the hard coat of melilot seeds.

The aim of this research was to develop a biological method for increasing seed germination and protecting grain crops against root rots caused by fungal phytopathogens.

3.2 Materials and Methods

3.2.1 Isolation of Cellulolytic Bacteria

Cellulolytic bacteria isolated from the rhizosphere of grain crops served as objects of the study. These bacteria were isolated from soils of agricultural lands in the north of Kazakhstan. This region is the major grain-producing area.

For isolated cellulolytic bacteria, the liquid Hutchinson's medium was used. Wheat straw served as a source of cellulose. To prepare an enrichment culture, a certain amount of soil from the rhizosphere was placed in a liquid medium. Cultivation was carried out for 5–7 days on a shaker at a speed of 180 rpm and a temperature of 28–30 °C. Further, the organized Hutchinson's medium without a carbon source was poured into Petri dishes. A circle of a certain diameter was cut out of the filter paper and placed on the bottom of the dish. The filter was tightly pressed against the medium with sterilized tweezers. The 1:10 and 1:100 dilutions of the enrichment

culture were prepared. 2 ml of suspension was taken from these dilutions and applied to filter paper, evenly spreading over the entire surface. The closed Petri dishes were placed into a thermostat and kept for 12–14 days at a temperature of 28–30 °C. The microorganisms were selected from the holes formed on the filter paper by the loop. They were plated in Petri dishes with the agarized Hutchinson's medium containing 2% water-soluble cellulose (Na-carboxymethyl cellulose). The pure bacterial cultures were obtained.

The resulting cultures were grown on the liquid Hutchinson's and Gould-Dexter's media and solid Hutchinson's and MPA media. The temperature for culturing bacteria was 28–30 °C. The cultures were maintained in nutrient agar slants and regularly subcultured (Egorov 2006; Emtsev and Mishustin 2005).

3.2.2 Studies on Germinations Seeds and Growth-Promoting Activity of Cellulolytic Bacteria Under Model Laboratory Conditions

To study the ability to increase the seed germination and their growth-stimulating activity, the bacteria were grown in liquid Hutchinson's medium under shaking conditions at a speed of 180 rpm and a temperature of 28 °C for 5–7 days. Seeds of wheat, barley, and oats recommended for cultivation in Kazakhstan were used in the experiments. Seeds of cereals before sowing were inoculated with a bacterial suspension at a concentration of 10^6–10^7 cells/ml, for 2 h at 23 °C. The treated seeds were sown in the 450 ml growth vessels. The duration of the experiments was 30 days. The non-treated seeds served as controls. Vermiculite was used as a substrate for plant growth. To feed the seedlings, Knop's liquid medium was used. Prior to the experiment, the substrate and Knop's solution were sterilized; the plants were watered with the sterile tap water. All the experiments were carried out in seven replicates. The plant biometric parameters, such as the length of stems and roots, were measured after 30 days of growing (Posypanov 2007; Stepanov et al. 2012).

The model laboratory experiments on the effect of bacteria on the germination and development of the grain crop seedlings were carried out in a climatic chamber (Memmert HPP 750 Constant Climate Chamber, Germany). The humidity, illumination, and temperature values in the chamber corresponded to the average parameters in the spring period.

3.2.3 Studies on Antagonistic Activity of Cellulolytic Bacteria Against Phytopathogenic Fungi

To study the antagonistic activity of bacteria, the phytopathogenic fungi *Alternaria alternata*, *Bipolaris sorokiniana*, *Fusarium solani*, and *F. oxysporum* var. *orthoceras*, causing alternariosis and root rot grain crops, were used as test organisms. We have isolated phytopathogens from the damaged plants in the fields

of the northern areas of Kazakhstan 2013–2015. Czapek's and PDA media were used for the cultivation of phytopathogenic fungi.

The antagonistic activity was determined by measuring the growth inhibition zones of phytopathogenic fungi (Egorov 2005). The fungi were inoculated by the pour plate technique in a medium, melted and cooled to 40 °C, and poured into Petri dishes. After solidification of the agar, blocks were cut out of the medium. A suspension of bacterial cells with a certain concentration was poured into the resulting wells. The plates were incubated at a temperature of 28–30 °C in a thermostat for 6 or more days. The data were presented as the growth inhibition zones of phytopathogenic fungi.

3.3 Identification of Cellulolytic Bacteria

To determine the taxonomic position of cellulolytic bacteria, the classical microbiological methods based on studying the culture-morphological and biochemical characteristics and properties of bacteria (Holt et al. 1997) and molecular genetic techniques were used. The Sanger sequencing method was used to confirm the taxonomic position of cellulolytic bacteria (Kwan Soo Ko et al. 2004; Kazartsev 2013). Genomic DNA was isolated from the examined strains using the PureLink® Genomic DNA Kits (Invitrogen, USA). The DNA concentration in the samples was determined with the Qubit Fluorometer (Invitrogen, USA) using the scale for dsDNA HS. Sequencing of the bacterial 16S rRNA gene was performed on the automated sequencer 3500 DNA Analyzer (Applied Biosystems, USA) using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) according to the manufacturer's protocol (BigDye® Terminator v3.1 Cycle Sequencing Kit Protocol Applied Biosystems USA). Identification was carried out by analyzing sequences of the 16S rRNA gene fragment using universal primers: 8F (5'-AGAGTTTGATCCTGGCTCAG-3′) and 806R (5'--GGACTACCAGGGTATCTAAT-3′). The reaction mixture (30 μl) contained 3 μl of the 10x reaction buffer (Fermentas), 2.5 mM of $MgCl_2$, 0.2 mM of each deoxyribonucleoside triphosphate (dNTP), 10 pmol of each primer, and one unit of Maxima Hot Start Taq DNA Polymerase (Fermentas). PCR has performed in the Mastercycler proS (Eppendorf) thermal cycler. The reaction was started by incubating the mixture at 95 °C for 7 minutes, followed by 30 cycles consisting of 95 °C for 30 seconds, 55 °C for 40 seconds, and 72 °C for 1 minute. The final elongation was carried out at 72 °C for 10 minutes. The amplified product was separated in the1.5% agarose gel; the bands were stained with ethidium bromide and visualized in a UV transilluminator. The 1 × TAE buffer was used as a running buffer. The PCR product was purified using the CleanSweep™ PCR Purification Reagent (Thermo Fisher Scientific, USA). Sequencing of fragments of the 16S rRNA gene from bacteria was performed on an automatic sequencer 3500 DNA Analyzer (Applied Biosystems, USA) using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) according to the manufacturer's protocol (BigDye® Terminator v3.1 Cycle Sequencing Kit Protocol Applied Biosystems USA).

3.3.1 Studies of Cellulolytic Activity of Bacteria and Composition of Cellulase Complex

The cellulase activity of bacteria was determined in a solid medium with 0.1% Na-CMC and expressed in unit/ml (Tolchenov et al. 2009). The Mandels-Weber method was used to quantify the cellulase activity (Mandels and Weber 1969). The composition of the bacterial cellulase complex was established in the A. Bach Institute of Biochemistry, Russian Academy of Sciences (Moscow, Russia).

3.3.2 Study of the Nitrogen-Fixing Ability, Biosynthesis B-Group Vitamins, and Composition of Amino Acids of Cellulolytic Bacteria

The nitrogen fixing of bacteria was evaluated using the Agilent technology 6890B Gas Chromatograph, USA (Wang et al. 1994). B-group vitamins were determined by microbiological methods (Tolysbaev and Bisenbayev 1996). The quantitative and qualitative composition of free amino acids was established using the AAA 400 Amino Acid Analyzer (INGOS, the Czech Republic) according to the protocol attached to the user's guide. The bacterial biomass was measured nephelometrically with spectrophotometer PD-303 ("Apel", Japan) in optical density units (RODU), calculated per absolute dry mass (a.d.m.) using the calibration curve, and expressed in g/1000 mL.

3.4 Statistical Analysis

The experiments were carried out in 5–7 replicates. Statistical significance of the obtained results was determined by the Student's t-test for the confidence probability with $p < 0.01$ (Urbach 2005).

3.5 Results

From the rhizosphere of grain crops, more than 50 isolates of cellulolytic bacteria were isolated. Of these, 23 cultures were selected, presumably with the ability to increase the germination of grain seeds. We checked the phytotoxicity of these cultures to the grain crop seedlings. After the testing, six cultures which showed no phytotoxicity and possessing growth-stimulating ability were selected.

For selecting the most active bacterial strains, the model experiments for studying their effect on seed germination and grain growth were carried out under laboratory conditions in a climatic chamber. Seeds of wheat, barley, and oats were used in the experiments. Seeds were inoculated with bacterial suspensions at a concentration of 10^6–10^7 cells/ml. The duration of the experiments was 30 days. The non-treated seeds served as controls. The data obtained in the study are presented in Table 3.1.

Table 3.1 Effect of cellulolytic bacteria strains on the germination and growth of cereals

Strains	Germination (%)	Shoot length (cm)	Root length (cm)
Wheat			
Control	66.1 ± 1.1	15.9 ± 0.1	9.3 ± 0.1
150	75.1 ± 1.3	17.4 ± 0.1	10.3 ± 0.1
121 N	82.3 ± 1.9	17.7 ± 0.1	11.2 ± 0.1
60(5)4	80.4 ± 1.8	17.2 ± 0.2	10.1 ± 0.1
21AS	90.1 ± 2.,1	18.2 ± 0.3	13.2 ± 0.1
60CS	88.3 ± 2.2	18.4 ± 0.2	13.1 ± 0.2
160	76.3 ± 1.2	17.5 ± 0.1	11.0 ± 0.1
Barley			
Control	60.2 ± 1.3	16.2 ± 0.1	10.8 ± 0.1
150	65.1 ± 1.3	17.4 ± 0.1	12.3 ± 0.1
121 N	69.3 ± 1.1	16.9 ± 0.1	12.2 ± 0.1
60(5)4	65.4 ± 1.3	17.6 ± 0.2	13.1 ± 0.1
21AS	78.0 ± 1.3	18.2 ± 0.2	14.9 ± 0.2
60CS	78.2 ± 1.4	18.3 ± 0.2	15.1 ± 0.1
160	70.3 ± 1.0	17.1 ± 0.1	12.0 ± 0.1
Oats			
Control	58.7 ± 1.1	15.3 ± 0.3	7.6 ± 0.1
150	65.1 ± 1.1	17.4 ± 0.1	8.8 ± 0.1
121 N	75.3 ± 1.0	15.9 ± 0.1	8.7 ± 0.1
60(5)4	73,4 ± 1.3	16.2 ± 0.2	8.1 ± 0.1
21AS	76.0 ± 1.3	17.4 ± 0.1	9.6 ± 0.2
60CS	75.3 ± 1.2	17,2 ± 0.1	9.8 ± 0.1
160	72.3 ± 1.1	16.0 ± 0.2	8.5 ± 0.3

It was established that all strains increased the germination of grain seeds by 8–36% (depending on the strain) and increased the growth of seedlings. The length of stems and roots increased by 4–19% and 6–42%, respectively, as compared with the control. Of the six strains studied, two bacterial strains 21AS and 60CS were selected which most actively improved seed germination and growth of grain crop seedlings.

We have examined the biocompatibility of these strains. It was established that the strains are not antagonists, but positively affect each other. At their joint cultivation, the rate of biomass accumulation is 28–31% higher than that of monocultures. It was also shown that the joint use of strains significantly enhances the effectiveness of their impact on grain crops. Inoculation of seeds with the strain association improved germination to 80–92% and increased the length of stem and roots by 24.5% and 45.3%, respectively, as compared to the use of monocultures.

The selected strains were examined for their antagonistic activity against phytopathogenic fungi. The following fungi that cause root rots in grain crops were used as test cultures: *Alternaria alternata*, *A. tenuis*, *Bipolaris sorokiniana*, *Fusarium solani*, *F. oxysporum*, and *F. oxysporum* var.*orthoceras* (Table 3.2).

Table 3.2 Antagonistic activity of bacteria against phytopathogenic fungi

Fungi	The diameter of the zone of inhibition (mm)		
	Strain21AS	Strain60CS	Association
A. alternate 28M	48.4 ± 2.1	45.3 ± 2.1	51.5 ± 2.2
A. tenuis 64S	39.1 ± 1.3	36.2 ± 1.3	42.4 ± 1.2
B. sorokiniana ET	26.2 ± 2.5	21.6 ± 2.5	40.1 ± 2.4
F. solani C11	48.5 ± 2.2	41.5 ± 2.2	50.3 ± 2.0
F. oxysporum M2	29.1 ± 1.4	23.3 ± 1.2	32.3 ± 1.5
F. oxysporum var. *orthoceras 18S*	13.3 ± 1.0	11.1 ± 1.1	17.8 ± 1.2

Values are means of five replications

Table 3.2 shows that cellulolytic bacteria were potent inhibitors of fungal phytopathogens. The strains of bacteria most strongly inhibited the growth of *A. alternate*, *A. tenuis*, and *F. solani* (diameter of the inhibition zone of 48–36 mm), somewhat less of *B. sorokiniana* (21–26 mm) and *F. oxysporum* (23–29 mm), and much less of *F. oxysporum* var. *orthoceras* (up to 13 mm). The data in Table 3.2 shows that the bacterial association inhibited the growth of phytopathogenic fungi more actively than that of monocultures. The zone ingibition of the fungi *F. oxysporum* var. *orthoceras* for association was 17.8 mm, for strains 21 AS and 60CS – 11.1 and 13.3 mm, respectively.

To identify strains of cellulolytic bacteria, their cultural-morphological and biochemical characteristics and properties were studied. It was found that the strain 21AS belongs to the genus *Bacillus* and the strain 60CS to the genus *Cellulomonas*. The sequencing according to the procedure described in the Materials and Methods section was performed to confirm the taxonomic position of cellulolytic bacteria. The resulting nucleotide sequences were added to the BLAST program and compared to the existing database. In this case, a similarity was found to the bacteria belonging to the genera *Bacillus* and *Cellulomonas*. Phylogenetic analysis for species identification of the strains belonging to the genera *Bacillus* and *Cellulomonas* was carried out by comparing them with 16S rRNA gene sequences of related bacterial strains from the NCBI database. Phylogenetic trees were constructed with the MEGA 6.0 software, using the neighbor-joining clustering method for calculating genetic distances (Fig. 3.1).

The results of phylogenetic analysis of the 16 s rRNA gene showed that the strains *Bacillus halodurans* C-125 and *Paenibacillus amylolytic* NBRC 15957 (Fig. 3.1a) were phylogenetically the closest to the strain *Bacillus cytaseus* 21AS. In the dendrogram, the strain 60CS was assigned to the genus *Cellulomonas* and defined as *C. flavigena* (Fig. 3.1b). Comparison of the 16S rRNA gene nucleotide sequences against the reference strain *Cellulomonas flavigena* DSM 20109 from the NCBI database showed a 99% homology.

The strains were deposited with the All-Russian Collection of Industrial Microorganisms (Russia, Moscow). They were assigned the following numbers: *Bacillus cytaseus* VKPM V-4441 and *Cellulomonas flavigena* VKPM V-4465.

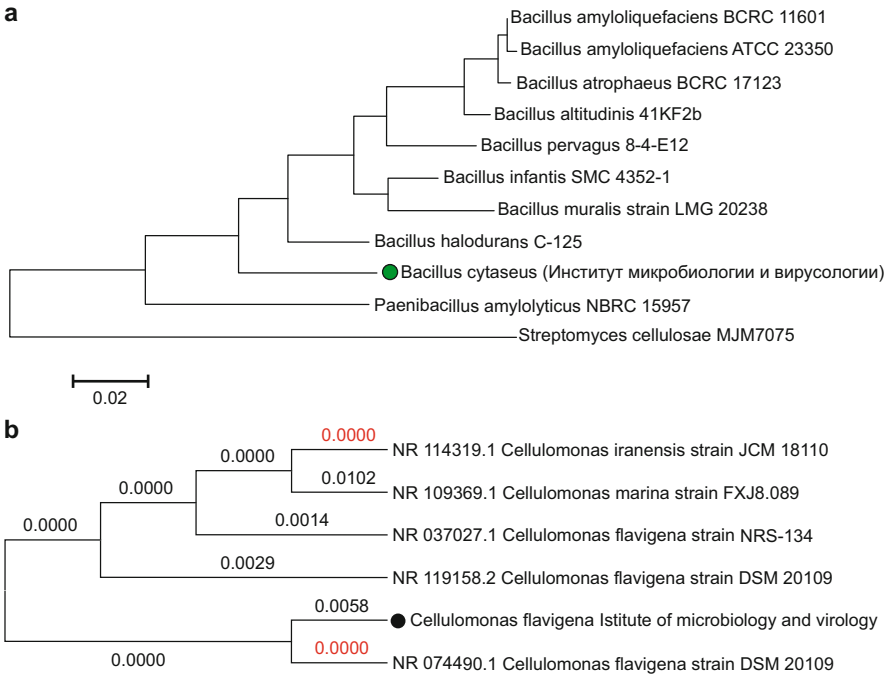

Fig. 3.1 Phylogenetic trees of genus *Bacillus* (**a**) and genus *Cellulomonas* (**b**)

The studies on the composition of the bacterial cellulase enzyme complex showed that the cellulase complex of *B. cytaseus* 21AS consisted of three enzymes: endo-1.4-β-glucanases, cellobiohydrolases, and β-glucosidases. The cellulase complex of *C. flavigena* 60CS contained two enzymes: endo-1.4-β-glucanase and cellobiohydrolase.

A detailed study showed that the association effectively increased seed germination by synthesizing cellulase enzymes. The ability of strains to actively stimulate plant growth is due to the fact that they synthesize biologically active substances, such as B-group vitamins, amino acids, and probably gibberellins. In addition, the strain *B. cytaseus* 21AS fixes the molecular nitrogen of the atmosphere, which creates additional nitrogen nutrition for plants (Table 3.3.).

The mechanism of action of the strains has been examined: after inoculation of the seeds, the bacteria colonize the hard seed coat, begin to grow, using cellulose of the hard seed coats as a carbon source; for this purpose, cellulase enzymes are synthesized that partially degrade the coat. Microholes and microcracks are formed on the seed coat. The seed coats become less strong, and through it, the transport of water and mineral and nutrient substances dissolved in it to the seed germ increases. This leads to an increase in germination and stimulation of seedling growth. We have named this process "biological scarification." The changes in the coating structure are clearly visible on micrographs of the wheat seed coats under the effect of the association of cellulolytic bacteria (Fig. 3.2).

Table 3.3 Biosynthesis of B-group vitamins and nitrogenase and cellulase activity by strains cellulolytic bacteria and association

Variants	Vitamins (mkm/g a.d.m.*)				Nitrogenase activity (mkmolC_2H_4/ml/h)	Cellulase activity (unit/ml)
	B_1	B_3	B_6	Pp		
Strain 21AS	18.4 ± 0,2	19.8 ± 0.2	14.6 ± 0.2	170.4 ± 2.2	3.87 ± 0.01	4.7 ± 0.01
Strain 60CS	15.2 ± 0,2	0	12.6 ± 0.2	168.6 ± 2.2	–	3.8 ± 0.01
Association	21.6 ± 0,2	43.8 ± 0.2	21.4 ± 0.2	235.0 ± 2.2	6.25 ± 0,02	5.3 ± 0.02

Values are means of five replications; a.d.m.* – absolutely dry mass

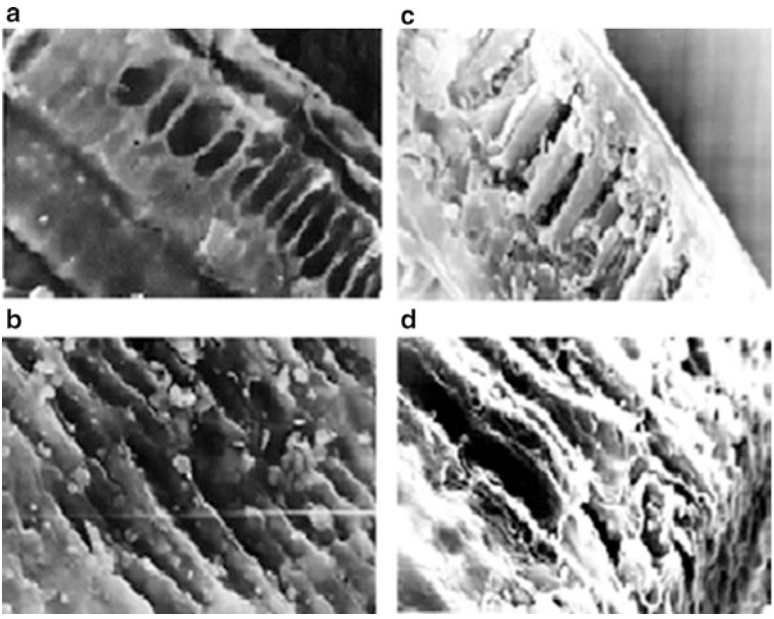

Fig. 3.2 Electron micrographs of hard coat seeds of wheat (1) and barley (2): a,b – control (without inoculation), c,d – after inoculation seeds

To study the effectiveness of the association under production conditions, the field trials of the bacterial association have been conducted in the north of Kazakhstan (Kostanay region) for 2 years. Spring wheat and barley seeds were used in the experiments. Seeds were subjected to pre-sowing treatment with the bacterial association. The non-treated seeds were used as the control. The agricultural chemical Biosil served as a reference (application rate of 0.05 L/t). The agrotechnology, generally accepted for this agricultural zone, was used in the experiments. Field trials have confirmed the high effectiveness of the bacterial association (Table 3.4).

The data in Table 3.4 show that the pre-sowing inoculation of grain crop seeds increased their germination up to 80–89% (61–65% in the control). The number of plants affected by root rot decreased 2.4–7.3 times. The grain crop yield increased by 2.6–3.2 centner/ha as compared to the control.

3.6 Discussion

The chemicalization of agriculture causes a decrease in immunity of agricultural crops, the emergence of resistant forms of phytopathogenic microorganisms, disturbance of soil microflora, and reduction of soil fertility. All this leads to a deterioration in food quality and adversely affects human health.

Table 3.4 Effect of inoculation seeds by the association of cellulolytic bacteria on germination and grain yield of wheat and barley

Variants	Germination (%)	Root rots (%) by growth phases		Grain yield (c/ha)
		Tillering	Ripeness	
Wheat				
Control	65.4	3.6	21.5	18.3
Biosil	80.2	2.3	14.5	20.7
Association	89.3	1.5	9.0	21.5
Barley				
Control	61.2	12.9	65.8	11.3
Biosil	83.8	10.5	12.6	16.8
Association	88.9	5.2	8.8	17.9

Values are means of eight replications

The problems of agriculture are solved in different ways. Some farmers improve the chemical plant protection products, continuing to have a disastrous effect on the environment, while others develop biological farming methods based on the use of preparations containing natural microorganisms (Bessonova and Mereshchenko 2014; Heusinkveld et al. 2016; Curutiu et al. 2017).

Our studies are related to the application of PGPRs in order to control phytopathogens and increase seed germination of agricultural crops. PGPRs can reduce the use of chemicals, improve yields, lower the production costs, and make the final products environmentally clean (Beneduzi et al. 2012; Ahemada and Kibretb 2014; Wang et al. 2014).

To increase seed germination, various methods are used in agriculture, including physical, chemical, and mechanical (Ning et al. 2014; Zin et al. 2016; Kataria et al. 2017; Ruttanaruangboworn et al. 2017). Scarification is the most commonly used method, that is, a mechanical violation of the seed coat integrity. This method often causes damage to the seed embryos, which leads to their infection with pathogens and a decrease in germination. In addition, scarification is an energy-intensive process and requires significant energy and labor costs (Behlyarova 2009; Chaodumrikul et al. 2016; Wagner et al. 2017).

We have developed a biological method for increasing the germination of cereals. The method makes it possible simultaneously to increase the germination of seeds and to protect the cereals from root rot. The method is based on the application of the association of cellulolytic bacteria. The use of cellulolytic bacteria in our studies is explained by the fact that the selected strains of the association have a wide range of desirable properties for use in agro-biotechnology:

- Are able to carry out "biological scarification" of seeds – partially destroy the hard seed coat, thereby increasing the access for water and nutrition to the seed embryo.

- Inhibit the growth of phytopathogenic fungi, reduce the damaging effects of root rots on grain crops seven times, and improve the phytosanitary situation in the soil.
- Stimulate the growth of plants due to the synthesis of biologically active substances (B-group vitamins, free amino acids, and probably gibberellins).
- Fix atmospheric nitrogen and increase its availability to the root of plants.
- Closely interact with plants and form an "associative symbiosis."
- Since cellulose is the bulk of plant residues in the soil, the size of this bacterial group is more significant as compared with other soil groups of microorganisms. Cellulolytic bacteria are frequently detected in the rhizosphere of agricultural crops, and bacteria with useful properties for agriculture can be found among them (Rasmussen et al. 2002; Naplekova 2010; Talia et al. 2012; Hong-Sheng Wu et al. 2017).

Bacteria are developing on the cellulose-containing substrates in the soil, destroy them, and improve the physicochemical properties of the soil and its structural state. The soil is supplied with organic matter, and the humus formation is intensified. All these processes have a positive effect on soil fertility.

The use of biological preparations based on this group of bacteria is environmentally safe, because it is based on the interaction of organisms in nature and does not lead to a disturbance of the biological equilibrium in the soil. Cellulolytic bacteria are widely distributed in nature, being nontoxic and nonpathogenic to humans, warm-blooded animals, and bees. Cellulolytic bacteria themselves as a natural factor are involved in improving soil fertility. The production created on their basis will not serve as a source of environmental pollution.

In conclusion, a biological method for increasing seed germination and protecting grain crops has been developed on the basis of the association of cellulolytic bacteria. The method enables to increase the germination to 80–89%, reduce the number of plants affected by root rot 7.3 times, and improve the yields of grain crops by 2.6–3.2 centner/ha. On the basis of this method, we have developed a new biological preparation "Phytobatsirin" to increase seed germination and their protection against root rots. Patents of the Republic of Kazakhstan have been obtained for the association of cellulolytic bacterial strains and method of producing the biopreparation in industrial conditions. Currently, the biopreparation is produced in the factory at the Institute of Microbiology and Virology and successfully used by the farming enterprises.

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