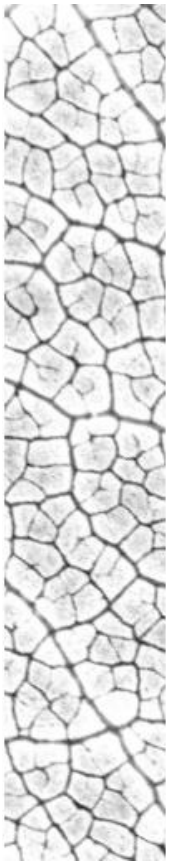

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Biological disinfection of sugar beet at storage bacteria-antagonists of pathogens of sugar beet root rot

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Abstract
The search for antagonistically active cultures of bacilli to fungal and bacterial pathogens of sugar beet root rot was carried out. Mycelial fungi are represented by genera such as *Aspergillus spp., Fusarium spp., Penicillium spp., Alternaria spp., Mucor spp*. et al. The study involved 44 cultures of bacilli to 10 microscopic fungi, 5 bacteria, 5 yeasts and 5 enterobacteria. We selected 14 working bacilli for further work, which have good antagonistic properties.

Keywords: sugar beet, kick rot, storage, microorganisms, bacteria, lactobacilli, microscopic fungi, yeast, enterobacteria, antagonistic activity, biocompatibility

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INTRODUCTION

Significant losses of sugar beet (*Beta vulgaris L.)* at storage are the activity of microorganisms that cause rotting of root crops. A comprehensive study of the symptoms of rotting sugar beet root crops shows that this is a multifactorial disease, in the manifestation of which many types of soil microflora are involved. Almost always, the defeat of root crops in clamps is caused not by a single fungus or bacterium, but by their complex, so the loss of sugar and the degree of rotting increase (The situation with sugar production in Kazakhstan was called catastrophic, Maui and Kozhabayev 2013, Dvorkina 1992).

The main causative agents of clamp rot are *Botrytis cinerea, Fusarium sp., Penicillium sp., Alternaria alternate, Oospora betae, Verticillium sp., Bacillus sp.* et al. (Stognienko and Vorontsova 2015). Infection occurs even in the field and continues to develop in clamps, causing weight loss, a decrease in food and technological value, and the death of root crops (Sviridov and Kolomiets 2012).

Clamp rot that primarily affects weakened roots: dry-cured, frozen, wounded, and damaged leaves and roots during the growing season (Maui and Kozhabayev 2013).

Sugar beet crop losses from these diseases in many regions of the world average from 5 to 20% (The situation with sugar production in Kazakhstan was called catastrophic). In some sugar factories, losses were up to 30%. When the amount of rotten mass mixture is 8-10% or more, plants often do not get crystal sugar at all. If the mother beet is affected at storage, the yield of suitable root crops and their productivity are significantly reduced by 15-60% (Maui and Kozhabayev 2013). It was found that root rot in the South-East of Kazakhstan is one of the most harmful diseases of sugar beet. The decrease in the yield of root crops, depending on the variety and the saturation of the crop rotation, is on average 40-60%, and the sugar content is on average 30-40% (The situation with sugar production in Kazakhstan was called catastrophic, Aitbayev et al. 2014).

So, sugar beet diseases are a factor in a significant reduction in the yield and deterioration of its quality.

The complex of protective measures includes treatment of beet plants from pests and diseases during the growing season, protection from mechanical damage during harvesting, transportation and loading, protection from freezing and drying, thorough rejection before laying in clamps, periodic monitoring of stored root crops, removal of centers of rot (Sugar beet, Sviridov 2014).

Much attention is paid to the organization of protective measures aimed at suppressing the activity of pathogenic microflora in clamps. For this purpose, chemical agents are traditionally used, but the use of these drugs leads to contamination of root crops with residual amounts of pesticides, as well as to a decrease in their commercial qualities, which initiates the search for alternative methods of protection (Sugar beet).

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Using the method of biological control of phytopathogens, as an alternative to the chemical method, allows for effective protection of root crops and obtaining environmentally safe products based on cultures of microorganisms. Their basis is antagonism. However, currently there are no registered bio-drugs in Kazakhstan to protect sugar beet from diseases at storage, and the use of imported drugs that are not adapted to the specific composition of the causative agents of clamp rot, characteristic of local climatic conditions, is not always effective.

In this regard, the chosen topic of the article is relevant and perspective.

RESEARCH MATERIALS AND METHODS

Object of Research

- microorganisms isolated from stored sugar beet: fungi, bacteria, yeast;
- collection microorganisms: LAB and bacteria of the genus *Bacillus*.

Methods for evaluating antagonism (Netrusov et al. 2005, Skorodumov et al. 2005).

Block method

The test culture of bacteria is sown in depth in a nutrient agar, in a Petri dish, and incubated in optimal, strictly observed conditions for the formation and accumulation of inhibitory compounds in the agar. Plating on the surface of the agar plate can be done. Then, with a sterile cork drill, cut out an agar disk (block) with the grown culture of bacteria and place it in another Petri dish on the surface of the agar medium that has just been sown with the culture of pathogen. The plate is kept for 30 minutes in the refrigerator (to avoid premature growth of the pathogen) to diffuse inhibitory compounds from the block into the agar thickness with the pathogen, then incubated under certain conditions optimal for the latter. The degree of antagonistic activity of the test bacterium is judged by the size of inhibition zone of the pathogen growth around the agar block.

Well method

In the medium, by the cork drill cut well with a diameter of 5-7 mm and in it is placed a certain number (for example, 0.2 cm^3) of liquid or semi-liquid medium with grown culture of the test antagonist. Do not make wells until the end, so that the culture fluid does not flow under the medium, which distorts the result. The plate is kept in the refrigerator for diffusion of inhibitory substances from the well into the agar thickness, then - in the thermostat for growth of the pathogen, after which the inhibition zone of the antagonist around the well is measured.

Stabbing method

Plating one loop of daily culture in the thickness of the agar plate containing the pathogen. Then incubation and the result reading (Vegas et al. 2006, Alemayehu et al 2016).

Method for evaluating of the maximum indicator of viability (Irkitova et al. 2012)

We estimate the viability indicator of microorganism cultures using the Miles&Misra method.

We estimate the indicator of the zhsp of microbial cultures using the Miles&Misra method.

Preparation of dilutions:

- preparation of the working suspension: the culture was washed off in a separate tube with a nutrient broth suitable for the culture;
- to prepare dilutions, sterile tap water or saline solution was poured in 9 ml;
- 1 ml of the initial suspension was added into the first tube – this is the 1st dilution (10^{-1});
- titration was carried out to 10^{-12} degrees.

For the preparation of each dilution, it is necessary to use a new pipette. Ignoring this rule leads to an erroneous result.

Plating on medium and registering results. The outer back of the Petri dish was divided into eight equal sectors. A precisely measured volume of bacterial suspension (20 µl) was applied to the surface of the medium using a sterile pipette (tip). Sowing on a dense medium was carried out, as a rule, from the last eight dilutions. Plating was made with different pipettes (tips). The pipette is held vertically. Drops do not rub, their area can be increased by slightly swaying the Petri dish. The cultures are incubated and then the number of colonies grown is determined. After plating, the Petri dishes are placed in the thermostat with the lids down. Incubation.

Cell counting. The number of cells in 1 ml of the test substrate is calculated using the formula 1:

$$M = a \times 10^n \times V \times 50 \quad (1)$$

where M - the number of cells in 1 ml;

a - the average number of colonies when sowing the dilution from which the sowing is made;

10^n - the dilution factor;

V - volume of the suspension taken for plating, in ml;

– the conversion factor from µl to ml.

The study of morphological, cultural, biochemical, and physiological characteristics of strains of microorganism cultures was carried out using generally accepted methods in bacteriological practice (Netrusov et al. 2005, Egorov 1985, Holt et al. 1997, Labinskaya 1978).

Genotyping of active cultures of microorganisms

DNA isolation was performed using the method described by Kate Wilson, which allows efficient isolation of DNA from gram-negative and gram-positive bacteria (Vegas et al. 2006).

Transfer 1.5 ml of the bacterial culture grown on a liquid medium to a centrifuge tube. Centrifuge for 2 minutes at 12 000 g. Remove the supernatant. Repeat

Fig. 1. Obtaining pure cultures of microorganisms

this step several times until a precipitate with buckwheat grain is formed. Resuspend the precipitate in 500 µl of STET solution. Add 30 µl of Lysozyme (10 mg/ml), gently pepetize and incubate at 37°C for 2 hours, shaking every 15 minutes. Add 30 µl of SDS solution and 3 µl of proteinase K. Mix thoroughly and stand overnight at 37°C, then another 2 hours at 50°C. Add 100 µl of 5 M NaCl and mix thoroughly. Add 80 µl of CTAB/NaCl solution, mix thoroughly, and incubate for 10 minutes at 65°C. Cool the test tubes. Add about 750 µl of chloroform/isoamyl alcohol, mix thoroughly, and centrifuge for 5 minutes at 12 000 g at room temperature. Carefully select the viscous aqueous (upper phase) supernatant in the new microcentrifuge tubes, do not affect the interphase. Add an approximately equal volume (400-600 µl) of chloroform/isoamyl alcohol, mix thoroughly, and centrifuge for 5-15 minutes at 12 000 g at room temperature. Collect the supernatant into a new microcentrifuge tubes. Add 0.6 volumes of isopropanol to precipitate the nucleic acids. Mix well, turning the test tube over until the DNA strands form a clearly visible residue. Centrifuge for 10 minutes at 12 000 g at room temperature, remove the supernatant. Wash twice the DNA precipitate 500 µl of 70% ethanol, and centrifuge as in the previous step. Carefully remove the supernatant and dry the DNA residue, leaving the lids open for 15-30 minutes. Dissolve the DNA precipitate in 100 µl of TE buffer. Dissolution can be accelerated by heating to 60°C for 10-30 minutes.

Store DNA for regular use in small aliquots at 4°C (24-48 hours).

Amplification of the 16S rRNA gene fragment. The PCR reaction was performed with universal primers [62] 8f 5' - AgAgTTTgATCCTggCTCAg-3 and 806R-5' ggACTACCAgggTATCTAAT in a total volume of 30 µl. The PCR mixture contained 25 ng of DNA, 1 Unit of Maxima Hot Start Taq DNA Polymerase (Fermentas), 0.2 mM of each dNTP, 1 PCR buffer (Fermentas), 2.5 mM of MgCl2, 10 pmol of each primer. The PCR amplification program included a prolonged denaturation of 95°C for 3 minutes; 32 cycles: 95°C for 30 seconds, 55°C for 40 seconds, 72°C for 60 seconds; and a final elongation of 10 minutes at 72°C. The PCR program was performed using the GeneAmp PCR System 9700 amplifier (Applied Biosystems).

Determination of the nucleotide sequence. Purification of PCR products from unbound primers was carried out by the enzymatic method using Exonuclease I (Fermentas) and alkaline phosphatase (Shrimp Alkaline Phosphatase, Fermentas) (Werle et al. 1994).

The sequencing reaction was performed using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applide Biosystems) according to the manufacturer's instructions, followed by fragment separation using the 3730xl DNA Analyzer automatic genetic analyzer (Applide Biosystems).

The resulting nucleotide sequence was compared with the nucleotide sequences of international databases.

Methods of storage

Storage of microorganisms was carried out according to generally accepted methods (Egorov 1985): subcultivation on slant agar media, under mineral oil, by cryopreservation.

RESEARCH RESULTS

At the end of 2018, 220 pure cultures of microorganisms were obtained from sugar beet of the Aksu hybrid, Almalybak village, Karasay district (experimental field of KazRIACP) and beets from clamps of the Koksu sugar plant (28 samples): 116 bacteria, 92 microscopic fungi, 12 yeast (**Fig. 1**).

For 2019, 58 gram-positive bacteria (including 2 collection strains of the genus *Bacillus*), 5 enterobacteria, 42 microscopic fungi, 7 yeast, and 61 LAB were taken for research.

We conducted search and screening of antagonist cultures, and selected the most active ones – 14 cultures. The advantage of each culture was estimated by the numbers of antagonism, the number of suppressed cultures, and the viability indicator. Cultural-morphological and physiological-biochemical features in the amount of 35: 21 - LAB and 14 - bacilli were studied in active antagonist microorganisms. As a result of genotyping 20 cultures of antagonist bacteria, their generic affiliation was established: *Enterobacter, Lactobacillus, Pediococcus, Enterococcus, Staphylococcus, Arthrobacter, Bacillus, Brevibacterium.* The selection was made based on the similarity of morpho-cultural properties.

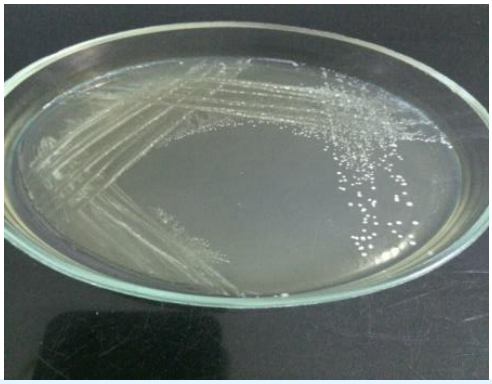

Fig. 2. Growth of the LAB colony on the MRS-4 medium

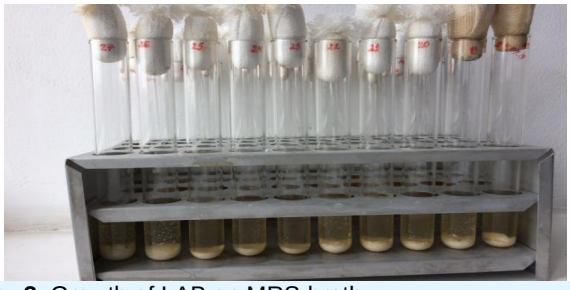

Fig. 3. Growth of LAB on MRS-broth

Study of morphological, biochemical, and physiological features of active antagonist cultures

Morphological characteristics of bacteria include the shape and size of cells, the location, presence or absence of spores, capsules, and the shape and location of spores.

Cultural characteristics were studied on dense and liquid nutrient media. On solid media the colonies are described. At sowing in a liquid medium, the amount of sowing material - the culture loop. A description of the growth pattern in the broth was performed based on the following characteristics: the presence of a wall ring; the presence, nature, thickness of the surface membrane; the nature and intensity of the turbidity; color, structure, and amount of sediment. The culture characteristics of LAB on a solid medium are shown in **Fig. 2**.

Some cultures are characterized by growth on MRS-agar in the form of surface round colonies with clear edges, white, shiny, sizes varied from small to large, the surface and edges are smooth - S-shape (**Fig. 2**). Others have colonies with uneven edges, pale gray in color, often with a compacted center.

When bacteria grow in the MRS-broth (**Fig. 3**), in some cases, a homogenous light turbidity is observed, in others flakes are formed, and the medium remains transparent. In all cases, a precipitate of greater or lesser intensity, loose, white in color, and of a fine-pored consistency is formed. So, growth in the broth is characteristic of all bacteria.

Fig. 4 shows variants of the microscopic picture of lactobacilli (a, b) on solid nutrient media. The studied culture is mainly represented by rods, differing in length,

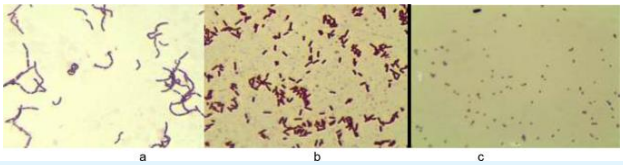

Fig. 4. Variants of the microscopic picture of the LAB (powered ×100)

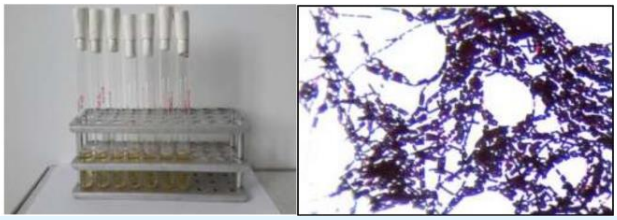

Fig. 5. Growth of spore-forming bacteria in a liquid nutrient medium and smear (powered ×100)

thickness and nature of the location, there are often rods, wrapped in rings. **Fig. 4a** – the rods placed by short and long chains, also have rings of bacteria; **Fig. 4b** – the rods placed one by one, two, clusters, bundles. There are also long thick rods arranged singly, sometimes in short chains. Cocci were also found among the bacteria (**Fig. 4c**).

In cases with other bacteria, the microscopy picture (powered ×100) gives the presence of gram-positive rods with rounded ends, arranged in a chain and clusters. There are spores of a central location, spherical or oval shape. This pattern is typical for both liquid and solid media.

On a solid nutrient medium, MPA colonies are dark beige in color, colonies of regular round shape, of various sizes, from small to large, flat, the surface is smooth, shiny, the edges are even, the structure is homogenous. Also sown colonies rounded irregular shape; large (more than 5 mm); convex; the surface is folded, wrinkled; the color of the colony is white, the edges are serrated - R-shape.

In the liquid nutrient medium, there is a turbidity of the medium, the precipitate does not fall out; a thin folded membrane is formed, slightly creeping on the wall of the test tube (**Fig. 5**).

There were also staphylococci, gram-positive cocci, growth on a solid medium - colonies of round, regular shape, yellow color.

The spectrum of studying the physiological and biochemical properties of bacteria was wide: proteolytic, lipolytic and amylolytic activity; fermentation of maltose, sucrose and mannitol; hemolysis, reduction of methylene blue, etc. (**Tables 1**, **2**).

When working with LAB, study the type of lactic acid fermentation; catalase, proteolytic, amylolytic activity; fermentation of maltose, sucrose, mannitol; dilution of gelatin; hemolysis, reduction of methylene blue (**Table 1**).

Table 1. Results of the study of the physiological and biochemical properties of LAB

Properties	Name of antagonist cultures																			
	LB2	LB3	LB4	LB6	LB11	LB12	LB18	LB19	LB22	LB25	LB26	LB28	LB29	LB32	LB39	LB42	LB49	LB54	LC84	L110	13LHB
Hydrolysis of starch	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hemolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fermentation of mannitol	-	-	-	-	±	±	±	±	+	-	+	±	-	+	±	±	+	+	-	+	-
Fermentation of sucrose	-	-	-	-	±	±	±	-	±	±	-	±	±	±	±	±	+	+	-	+	-
Fermentation of maltose	-	-	-	-	-	±	±	-	±	-	±	±	±	±	±	±	+	±	+	+	-
Liquefaction of gelatin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Reduction of methylene blue	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
Proteolysis of casein	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-
Lactic acid fermentation	-	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 2. Results of the study of physiological and biochemical properties of bacilli

Properties	Name of antagonist cultures													
	5B	6B	8B	9B	14B	17B	23B	29B	32B	36B	39B	40B	41B	B 154
Mobility	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase activity	±	+	±	±	+	+	+	+	±	+	±	+	+	±
Hydrolysis of starch (mm)	-	4	-	4	-	3	-	-	-	5	-	3	-	8
Reduction of methylene blue	+	+	-	±	-	±	-	±	-	±	±	±	±	+
Liquefaction of gelatin	-	+	-	-	-	+	-	+	-	+	-	±	-	+
Proteolysis of casein	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth in the broth	±	+	±	±	±	+	+	+	+	+	+	+	+	+
Hemolysis (mm)	-	2	-	-	-	-	-	2	-	2	-	2	5	5
Fermentation of mannitol (acid, gas)	ag	-	ag	ag	ag	a	-	a	-	a	a	a	a	a
Fermentation of sucrose	+	±	±	+	±	+	±	+	-	+	±	±	+	+
Fermentation of maltose (acid, gas)	+	-	ag	+	+	±	+	±	-	+	+	±	-	±
Phenylalanine test	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Growth at NaCl														
5%	-	+	-	-	-	+	-	+	-	+	+	+	+	+
7%	-	+	-	-	-	+	-	+	-	+	+	+	+	+
10%	-	-	-	-	-	±	-	±	-	-	-	-	±	±
Lipolytic activity	-	-	-	-	-	-	-	-	-	-	-	-	-	-

LAB do not have catalase activity, which was found in the reaction with hydrogen peroxide. None of the cultures showed the ability to hydrolyze starch, hemolysis of red blood cells (on 2% blood agar), and liquefaction of gelatin.

Our cultures have the ability to ferment mannitol, sucrose, maltose (13 cultures each), two cultures reduce methylene blue. The majority of lactic bacteria possess a proteolytic activity that was studied in the Aikman's environment.

After incubation in a culture thermostat, the producing proteolytic enzyme causes peptonization of milk protein - casein, as a result of which transparent zones are formed around such colonies that clearly stand out against the common milky cloudy background of the medium. The casein hydrolysis zone was measured in millimeters from the edge of the stroke or colony to the border of the light zone. The larger the light area, the higher the caseinolytic activity of bacteria.

The size of zones varied from 2 to 7 mm, with an average of 4.7 mm.

The main characteristic is the type of lactic acid fermentation.

Lactic acid fermentation is a type of fermentation in which the final product is lactic acid.

Depending on which products are formed at glucose fermentation - only lactic acid or other organic products and CO_3, LAB is usually divided into homofermentative and heterofermentative. This division reflects the fundamental differences in the pathways of sugar catabolism. It is also necessary for their identification.

To detect gas formation, the studied LAB cultures were sowed by the stabbing method with a bacteriological loop into a test tube with 5 ml of semi-liquid 0.7% lactic bacterial agar (factory medium) and 1 ml of starvation agar was poured on top. The ability to gas formation was judged after 24 hours in a thermostat at 39°C, by the formation of gaps in the agar column in tube by the evolution of carbon dioxide.

The main differentiating feature is the carbon dioxide evaluation, which is formed in heterofermentative bacteria at glucose fermentation.

As a result of gas formation evaluation, 3 cultures ferment lactic acid to carbon dioxide, namely LB3, LB6, LB19, i.e. these are heterofermentative cultures.

Fig. 6 shows hetero- (left - bubbles) and homofermentative lactic acid fermentation.

These characteristics correspond to the description of the LAB for the bacterial determinant [60]. Positive enzymatic activity (proteolysis) increases the effect of antagonism, so this fact will be taken into account when creating a drug.

For other bacteria (**Table 2**), the following physiological and biochemical properties were studied: catalase, proteolytic, amylolytic and lipolytic activity; fermentation of maltose, sucrose, mannitol; gelatine

Fig. 6. Hetero- and homofermentative fermentation

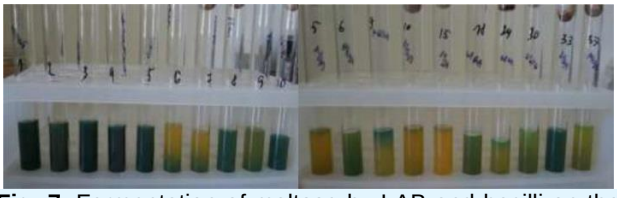

Fig. 7. Fermentation of maltose by LAB and bacilli on the Hiss medium

Fig. 8. Deamination of the amino acid phenylalanine by culture 40B

liquefaction; hemolysis; reduction of methylene blue; growth under anaerobic conditions; mobility; growth at NaCl (5, 7, 10%); reaction to phenylalanine.

Cultures are mobile and have catalase activity in reaction with hydrogen peroxide. None of the cultures showed the ability to casein proteolysis and lipolysis.

Our cultures ferment mannitol, sucrose, maltose, in some cases to form acid and gas; decompose methylene blue (3 cultures), starch (6 cultures), gelatin (6 cultures). In 6 cases, hemolysis of red blood cells occurs. According to the phenylalanine test, 1 culture – 40B is active.

The growth of microorganisms in different concentrations of table salt showed that some cultures grow well in all three solutions (4 cultures), and 4 cultures grow in concentrations of 5% and 7%.

The properties of active cultures have been studied in full sufficient volume. The results obtained can be used for identification of microorganism cultures, and will also add information about them.

The following photos show the properties we have studied, namely maltose fermentation (**Fig. 7**), using the

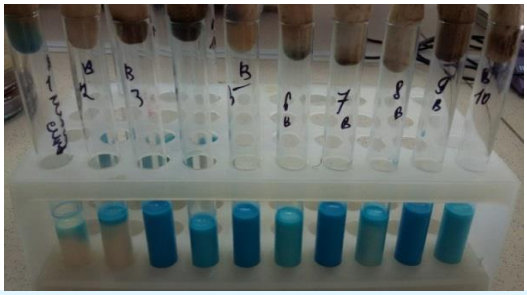

Fig. 9. Reduction of methylene blue of LAB in a medium with milk

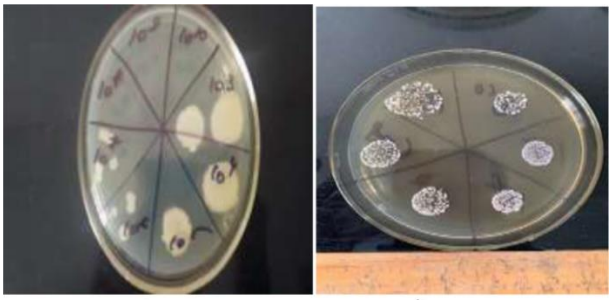

Fig. 10. Estimation of the maximum viability indicator of bacilli (a) and lactobacilli (b)

Hiss medium for this purpose; deamination of the amino acid phenylalanine in a phenylalanine test (**Fig. 8**); reduction of methylene blue in a medium with milk (**Fig. 9**).

In the future, we evaluated the maximum viability indicator of the antagonists.

The bacilli (**Fig. 10a**) gave numbers in the range of 10^5-10^7 CFU/ml, two cultures had 10^3 and 10^4 CFU/ml, perhaps this is due to the fact that the MPA medium does not meet the requirements of these cultures as food sources. Therefore, it is necessary to strengthen the prescription composition of the MPA or choose another rich medium, conduct seeding and then research to assess the viability indicator.

LAB (**Fig. 10b**) have a number of viable cells in 10^9-10^{10} CFU/ml, which was taken into account by sowing on solid culture media.

Cultures of microorganisms were stored by various methods in order to avoid their losses and their properties.

One of the methods is the method of subcultivation under mineral oil.

Yeast, fungi, and bacteria (aerobes) were grown on the surface of a short – sloped (at an angle of 45°C) medium, and lactobacilli (microaerophils and facultative anaerobes) were grown in a semi-liquid medium containing 0.2-0.3% agar. At the end of the incubation period, store in the refrigerator until the complete completion of the metabolism process. This depends on the time of incubation, for example, if the culture grows for a day, then it is necessary to keep the culture in the refrigerator for a day, after some exposure at room

Table 3. Antagonism of bacilli to fungi (mm)

№	Name of test cultures	Fungi – pathogens (mm)					
		As. №1, B16	Fus. №3, B13	Penic. purpur №4, B14	Fus. №6, B1	As.nig №8, B3	Penic №9, B7
1	6B	10	-	15	9	-	-
2	17B	-	-	10	15	-	5
3	23B	-	-	10	10	-	-
4	29B	-	10	5	12	7	10
5	36B	13	-	15	10	12	-
6	39B	-	12	-	-	12	15
7	40B	-	10	6	5	3	10

temperature to eliminate the influence of sudden temperature changes on the microbe.

The most suitable for filling cultures of microorganisms is highly purified medical vaseline oil with a density of 0.8-09. It is sterilized for 1 hour in a drying chamber at a temperature not higher than 150°C.

Cultures are filled with oil so that its layer is neither more nor less than 1 cm above the medium or the upper edge of the slope medium.

Storage of cultures is carried out at a temperature of +4-5°C during the year.

Active antagonist cultures were additionally stored by cryopreservation.

Microorganisms were grown under optimal conditions until the beginning of the stationary growth phase or the end of the formation of resting forms on a solid nutrient medium, the growth should be abundant. To protect cells from the damaging effects of low temperatures, they were previously suspended in cryoprotector solutions.

We used a cryoprotective medium containing 20% glycerol, 10% sucrose based on elective broth for this group of microorganisms.

A suspension of cells with a high density (10^9-10^{10} cells in 1 ml) was added into ampoules or vials with a screw cap (cryoprobe). The ampoules were placed in a refrigerator with a temperature of + 4-5°C for 30 minutes, then minus 20°C for a few hours and then stored in a low-temperature refrigerator at minus 77-80°C. In such conditions, it can be stored up to 5-10 years.

Working samples are stored using the subcultivation method on slope agar media. Storage of cultures is carried out at a temperature of +4-5°C for no more than 3 months.

When laying for storage, it is necessary to constantly monitor the viability indicator.

Genotyping of microorganism cultures

Identification of 20 cultures of antagonist bacteria was performed by determining the direct nucleotide sequence of the *16s rRNA* fragment of the gene, followed by determining the nucleotide identity with sequences deposited in the international Gene Bank database, as well as constructing phylogenetic trees with nucleotide sequences of reference strains.

The studied cultures were assigned by genotype to bacteria of the genus *Enterobacter, Lactobacillus, Pediococcus, Enterococcus, Staphylococcus, Arthrobacter, Bacillus, Brevibacterium.*

Results of Antagonism

According to literary sources, it is known that bacilli are the most perspective cultures against causative agents of clamp rot. Therefore, these cultures were used as antagonists. 54 lactobacilli were also studied for the same pathogens – fungi, yeast, and endobacteria, but they gave weak indicators of antagonism.

It was also concluded that the method of wells and blocks is more suitable for bacilli, and the stabbing method – for lactobacilli, where they gave positive results. In cases with the stabbing, the bacilli did not give a single positive result, if in cases with LAB, it was possible to detect manifestations of bacterial suppression using block and well methods.

At the 1st stage, antagonists were selected for mycelial fungi isolated from stored sugar beet as the main pathogens of sugar beet root rot.

Cultures of microscopic fungi were grown during the day on Petri dishes on Chapek agar at 28°C.

Depending on the method, wells were made and blocks were placed. Then the Petri dishes were left in the thermostat and the growth of fungi was observed every day for 7 days.

Used different methods of antagonism to work out the method and obtain a more accurate result, since different methods of sowing the antagonist are effective for different groups of microorganisms.

As a result of research, it was found that all bacteria have the ability to inhibit the growth of phytopathogenic fungi.

The results of the study of bacterial antagonism to phytopathogenic fungi are recorded in **Table 3** and **Fig. 11**.

Fig. 11 shows some positive results. The following bacteria showed the greatest activity in relation to fungi of the genus *Penicillium, Fusarium*: 6B, 17B, 36B, 39B.

Fig. 11. Antagonistic activity of studied test cultures of bacteria against phytopathogenic fungi

Table 4. Antagonism of bacilli to bacteria, yeast and endobacteria (mm)

№	Name of test cultures	Bacteria-pathogens (mm)						
		Bac. №1, mpa 1M	Bac. №3, 13B	Bac. №5, 32B	Yeast №7 1B/10	Yeast №9, 1M/5	Ent №12 B16/b 85E	Ent №14 1B/1
1	6B	9	-	20	-	-	15	1
2	14B	-	15	25	-	10	-	1
3	17B	-	5	12	-	-	-	5
4	23B	-	6	13	-	-	-	6
5	29B	-	-	7	12	-	-	3
6	36B	-	-	14	10	-	15	1
7	39B	-	-	25	-	15	-	1
8	B 154	-	-	25	-	20	-	2

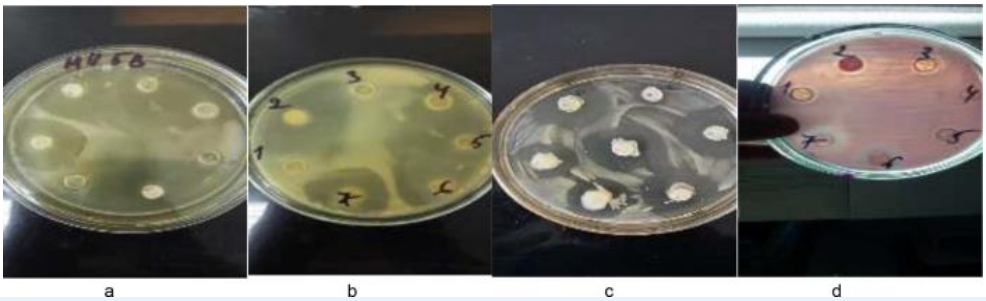

Fig. 12. Antagonistic activity of test cultures of bacilli under study against cultures of bacilli (a, b), yeast (b), enterobacteria (g)

At the 2nd stage, antagonists to bacteria and yeast isolated from stored sugar beets were selected.

As a result of the analysis of antagonistic manifestations of microbes-antagonists to fungal pathogens of sugar beet root rot, the most active cultures were selected for processing them on bacterial and yeast pathogens.

Also, the evaluation was performed using the method of blocks, wells and added another method of plating - stabbing.

The results of the study of bacilli antagonism to bacterial and yeast infections are presented in **Table 4** and **Fig. 12**. The following bacteria showed the highest activity in relation to 32B bacteria, yeast, and enterobacteria: 6B, 14B, 39B, and the collection strain B 154.

Thus, it was found that bacteria of the genus *Bacillus* significantly inhibit the growth of phytopathogenic fungi, bacteria, yeast, and endobacteria.

CONCLUSION

As a result of screening of antagonistically active bacilli, it was found that the main candidates for inclusion in the drug are bacterial cultures: 6B, 17B, 23B, 29B, 32B, 36B, 14B, 39B, 40B, 41B, 154, because they act on a large range of microorganisms and have high levels of antagonistic activity and suppress up to 6-7 pathogens of fungal, bacterial and yeast nature.

To create a complex drug against causative agents of clamp rot, several strains are required that can suppress the activity of phytopathogenic fungi, bacteria, and yeast as much as possible. For this purpose, we will evaluate the biocompatibility of 14 antagonistically active cultures of bacilli and create a domestic biological product based on strains isolated from substrates of local origin to protect sugar beets from rotting at storage, which is an urgent problem not only in Kazakhstan but also for other beet plants countries.

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