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Study of biological properties of *Lactobacillus helveticus* strains isolated in the Karaganda region for the design of the consortium

In the leading scientific centers of the world, research in the field of microbiology of dairy products for healthy nutrition production and probiotics containing live cultures of lactic acid bacteria, which are antagonists of various representatives of opportunistic-pathogenic and pathogenic microflora of the human intestinal tract, becomes particularly relevant. The effectiveness of probiotic preparations and products of milk functional nutrition depends primarily on the properties included in their species of different strains of bacteria. In this connection, at present the priority is given to the study of lactic acid strains isolated from natural sources, having high probiotic activity. One of the main components of starter cultures for dairy products and probiotic drugs is most often bacteria of the genus *Lactobacillus*. This article presents the study of morphological, culture properties, acid-forming ability, antibiotic sensitivity *Lactobacillus helveticus* isolated in the Karaganda region, having antagonistic activity towards test strains: *Staphylococcus aureus* NCTC 12973/ATCC ® 29213 ™, *Escherichia coli* NCTC 12923/ ATCC® 8739™, *Salmonella typhimurium* NCTC 12023/ ATCC® 14028™, *Pseudomonas aeruginosa* NCTC 12903/ ATCC® 27853™, *Klebsiella pneumonia* NCTC 9633/ ATCC® 13883™. The use of modern microbiological methods allowed screening of isolated cultures and selecting of biological active strains: *Lactobacillus helveticus-17, Lactobacillus helveticus-20, Lactobacillus helveticus-14, Lactobacillus helveticus-15.* According to the obtained results, strains *Lactobacillus helveticus* promising applicants for the consortium *Lactobacillus spp.* And it makes possible to judge the competitiveness of these strains.

Keywords: cultivation, strain, Gram color, *Lactobacillus* helveticus, antagonistic activity, acid formation activity, antibiotic sensitivity.

Introduction

Today the market of Kazakhstan uses various compositions of probiotic cultures to prepare probiotics and functional food products. The effectiveness of probiotic preparations and functional food products depends primarily on the properties included in their species of different strains of bacteria.

One of the main components of starter cultures for similar products is most often bacteria of the genus *Lactobacillus* [1].

Bacteria of the genus *Lactobacillus* are Gram-positive, non-pore-forming, fixed sticks, bond or optional anaerobes, with high enzymatic activity. They are very demanding to food sources and need rich complex nutrient environments. Acid resistance of lactic acid sticks is their hallmark. Lactic acid stick growth in alkaline and neutral medium is slowing down. The second distinctive feature of lactic acid sticks is their alcohol resistance. Lactic acid sticks are able to reproduce in nutritional substrates at high concentrations of alcohol.

Lactobacteria form round, smooth, convex with flat edges, opaque and non-pigmented colonies on nutrient medium. They well grow on the semi-fluid nutrient medium containing 0.15–0.75 % of concentration of an agar. The agar creates the low oxidation-reduction potential of the environment and microaerophilic conditions.

Lactic acid sticks differ in their biochemical and physiological properties. Lactic acid sticks have a brooding type of metabolism, cleave carbohydrates, and at least half of the carbon of the end products of fermentation is lactate. *Lactobacteria* are found in conditions of excess carbohydrates: for example, in foods (lactic acid products) and substrates of vegetable origin. In addition, they occupy many niches inside (normal phlora) and on the surface of the human body [2].

The importance to the organism of these bacteria lies in their metabolic functions; they can suppress the growth of various pathogenic and opportunistic microorganisms by creating an acidic medium due to the production of lactic and acetic acid, hydrogen peroxide, ethanol as a product, etc. [3].

Bacteria of the genus *Lactobacillus* have always attracted and attracted the attention of scientists and researchers around the world due to their great practical value. To date, the general biological properties of certain species of the genus *Lactobacillus* have been studied in detail by scientists and researchers abroad, CIS and Kazakhstan.

On the study of biological properties and correct identification of lactobacilli, the works of scientists and researchers in this field from Kazakhstan are devoted: K.Kh. Almagambetov, A.R. Kushugulova, I.S. Savitskaya, S.A. Saduahasova, etc. [4–5].

Although the study of the biological properties of lactobacteria strains cannot be considered complete:

– First, it is very likely that the biological properties of lactobacteria will change during long-term storage as industrial crops.

– Second, the physiological variability of human disease agents has often been observed recently, and many studies have shown an increasing virulent of opportunistic strains. Therefore, the antagonistic effects of lactobacteria on opportunistic strains may also change and require adjustments.

– Third, variability of opportunistic bacteria also affects the increase of antibiotic resistance. The produced resistance, supplemented by plasmid transmission to sensitive strains, occurs in bacteria faster than expected, a trend that undoubtedly affects lactobacteria as well. However, it is necessary to constantly study the antibiotic resistance of lactobacteria, as already existing antibiotics are constantly being improved and new antibiotics are being created.

– Fourth, lactobacteria are largely naturally resistant to a range of antibiotics, allowing them to be used as a probiotic in the process of antibiotic therapy. Since, when taking probiotics, lactobacteria enter into the human body in the state of antibiosis, it undoubtedly affects their biological properties [6]. Therefore, according to literary data, it was concluded that under the action of gastric juice and bile probiotics lose more than 90 % of their activity even before entering the intestine directly. It is a disadvantage which is considered insufficient resilience when exposed to factors such as temperature, bile salts, etc.

Therefore, the study and influence of various factors on the growth and biological properties of lactobacteria is relevant. The aim of the research is to study biological properties in order to design a consortium of lactobacteria isolated in the Karaganda region with the most optimal characteristics.

There were isolated 6 strains of lactobacteria from milk product (cheese, brynza, suluguni- cooked at home) produced in the Karaganda region (*in vitro* study). The tests were carried out in accordance with aseptics regulations.

Methodology

Sampling: At the intended sampling site, the surface of cheese, brynza, suluguni was burned by heated scalpel. A sterile probe was inserted obliquely into the middle of the head at 3/4 of its length. From a piece of cheese on the probe, 15 gm of cheese was taken with a sterile spatula and placed in a sterile petri dish. After 10 g of cheese was weighed on a petri dish, transferred into sterile porcelain mortar with a pestle, and thoroughly rubbed [7].

Study of morphological and cultural properties, mobility and test for catalase: Further, ten-fold dilutions from each product in sterile saline were prepared before sowing, followed by seeding on petri dishes with agar MRS medium. The plates were cultured at 37 °C for 2 days. After incubation, 18 strains of lactic acid bacteria were isolated from the milk product (cheese, brynza, suluguni), of which 6 isolated colonies were typical of lactobacilli, assessed through microscope (Gram coloring) and seeded on MRS broth. After 2 days of incubation control smears were made from all tubes with broth, after which to extract the isolated colonies by ten-fold dilution method, followed by seeding on Petri dishes with agar MRS medium. Crops were incubated at 37±1 °C temperature during 48 hours [8; 119].

After incubation, isolated colonies were determined with respect to Gram color, mobility, catalase presence and identified on *MALDI BioTyper*.

To determine the ratio of isolated strains to Gram color, smears were prepared from the colonies, stained by the Gram method, and microscoped using a digital ocular USB camera ToupcamTM Industrial digital camera, 14 Mpix.

Catalase Activity Test: Catalase activity of cultures was determined about the ability of catalase to decompose hydrogen peroxide with the release of gas bubbles. The reaction was set with a daily culture cooled to room temperature on a sterile slide. An isolated colony taken from the surface of the nutrient medium was rubbed on glass and a drop of 3 % hydrogen peroxide solution was pipetted. If gas bubbles appeared on the glass in 30 to 60 seconds, the reaction result was considered as positive. There was placed a test sample in parallel [9].

The mobility of the isolated cultures was determined by the "crushed drop" method [10; 7].

Grown cultures were identified using MALDI Bio Typer. Samples were prepared by direct transfer of fresh unit colony to polished steel target MSP 96 (Bruker Daltonik) and dried. The 1μl saturated a-cyano-4-hydroxy-cinnamic acid (HCCA) matrix solution was coated in 50 % acetonitrile — 2.5 % trifluoroacetic acid (Bruker Daltonik) and dried at room temperature [11].

Criteria of identification validity were judged by value of coincidence coefficient (Score values) — 2,300–3,000 — highly probable identification of species, 2,000–2,299 — reliable identification of genus, probable identification of species, 1,999–1,700 — probable identification of genus, 1,699–0 — identification failed.

The study of antagonistic activity of antagonist strains in relation of test strains to pathogenic and opportunistic microorganisms of different groups was determined by method of delayed antagonism. For research there were used test — stains: *Staphylococcus aureus* NCTC 12973/ ATCC® 29213™, *Escherichia coli* NCTC 12923/ ATCC® 8739™, *Salmonella typhimurium* NCTC 12023/ ATCC® 14028™, *Streptococcus pyogenes* NCTC 12696/ ATCC® *19615*™, *Klebsiella pneumonia* NCTC 9633/ ATCC® 13883™, *Pseudomonas aeruginosa* NCTC 12903/ ATCC® 27853™, *Streptococcus pyogenes* NCTC 12696/ ATCC® 19615™, *Candida albicans* NCPF 3179/ ATCC® 10231™ (stains taken from «Human microbiome and longevity «National Laboratory Astana" Nazarbayev University).

2-day cultures of lactic acid sticks grown on MRS-1 medium were looped onto Petri dishes with MRS-5 medium. After 2 days incubation at 37 °C, lactic acid stick strains were inhibited by UV rays for 30 minutes. Then, the surface of the plates was poured with a second thin layer of molten and cooled to +46 °C MRS-5 containing agar 0.7 % and mixed with the suspension test strains (0.1 ml of $1*10^9$ mCFU/ml bacteria test strain suspension) [12].

Antagonistic activity was judged by the zone of no growth of test strains around the colony of the tested strain lactobacilli: zero — at the width of the zone of no growth, low — 11–15 mm, average — 16–20 mm, high 21 mm and more. The study was performed in triplicate and the results were expressed as arithmetic mean.

Method involves extracting antibacterial factors — complex products, a component of which is a protein or polypeptide component responsible for bactericidal activity. After inhibition of bacteria by chloroform pairs or UV rays, semi-liquid agar with test cultures of pathogenic and opportunistic bacteria is laminated followed by incubation at 37 °C for 18–24 hours. Antibacterial substances delay the growth of test strains and a clear zone is recorded above the plaque of studied microorganisms on the background of continuous growth [13].

Determination of acid formation activity. Two tubes of each culture were removed and put into a refrigerator for rapid cooling to prevent further acid production. Then, 10 ml of culture liquid was added to glass flasks, and phenolphthalein was added as an indicator 1 drop.

The total acidity was determined by titration of decinormal alkali NaOH, which was added dropwise from the burette to the retorts with the poured culture liquid until a stable pink stain appeared. The amount of decinormal alkali that was used for titration corresponds to the amount of decinormal acid produced in 10 ml of culture liquid [14].

Data of acid formation activity, expressed in degrees Turner /°T/, was calculated by the formula:

$$°T = a \times k \times 10,$$

where a — is the number of milliliters of 0.1M caustic soda solution to be titrated; k — is correction to the titre of 0.1M caustic soda solution; 10 — is a correction factor for the mass of the analyzed sample.

The sensitivity of probiotic bacteria to antibiotics was determined by disk- diffusion test. From the test cultures, there were prepared the suspensions conforming to the optical turbidity standard of 5 units (with a microbial body content of about $1{,}5\times10^8$ CFU/ml), 1 cm^3 of the culture suspension was applied to each agar medium dish, uniformly distributed over the surface by lawn method and slightly dried in laminar flow. Further, the antibiotic discs of 5 pieces were applied to the surface of the nutrient medium seeded with a suspension

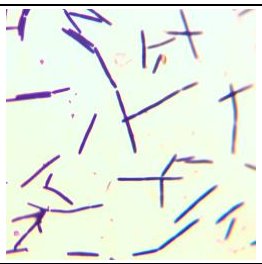
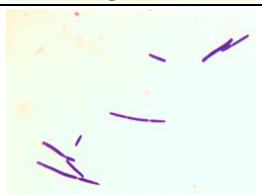
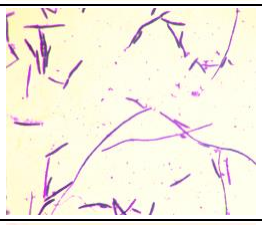
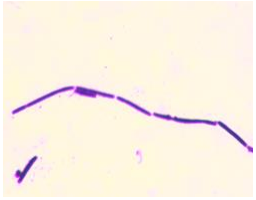
of lactobacteria cells. Inoculated plates with discs were incubated at 37±1 °C for 48 h. The antibiotic graph was formed by the diameter of the growth retardation zone of microorganisms. The study was performed in triplicate and the results were expressed as arithmetic mean.

Results and discussion

Studies carried out on cultural and morphological signs show that they belong to the genus *Lactobacillus* (Table 1).

Table 1

Identification of isolated strains by morphological properties, mobility and test for catalase

No.	Symbol of strains	Morphological characteristics of cells	The name of the strain after identification on MALDI-TOF	The microscopic drawings of the isolated strains
1	13	Gram positive and rod-shaped bacteria, non-motile, bacteria have no spores, cells are located single, in pairs, in clusters, or short chains. Catalase-negative	*Lactobacillus helveticus – 13*	
2	14	Gram positive and rod-shaped bacteria, non-motile, bacteria have no spores, single cells and in pairs. Catalase-negative	*Lactobacillus helveticus – 14*	
3	15	Gram positive large rods-shaped bacteria, non-motile, cells arranged in pairs or short chains. Bacteria have no spores, Catalase-negative	*Lactobacillus helveticus – 15*	
4	17	Gram positive, long thin rods-shaped bacteria, cells are located single, in clusters, or short chains. Bacteria have no spores, non-motile. Catalase-negative	*Lactobacillus helveticus – 17*	
5	20	Gram positive large rods-shaped bacteria, non-motile, cells arranged short chains. Bacteria have no spores, Catalase-negative	*Lactobacillus helveticus – 20*	
6	22	Gram positive, long thin rods- shaped bacteria, cells are located in clusters. Bacteria have no spores, non-motile. Catalase-negative	*Lactobacillus helveticus – 22*	

Using a digital ocular USB camera Toupcam™ Industrial digital camera, 14 Mpix, reproduced high-quality photomicrographs to create a photo atlas of probiotic cultures isolated in the Karaganda region.

Isolated strains on the second day at t –37 °C grow well on MRS nutrient medium (Table 2). These are optional anaerobe bacteria, micro aerophylles.

Table 2

Cultural characteristic of selected strains *Lactobacillus helveticus*

The name of the strain after identification on MALDI-TOF	Cultural characteristic of strain	Morphology of colonies
Lactobacillus helveticus – 13	Colonies on MRS agar colonies white, with uneven edges, are convex, 2–4 mm in diameter, non pigmented	
Lactobacillus helveticus – 14	Colonies on MRS agar colonies white small, medium colonies, with uneven edges, are convex, 1–2 mm in diameter, non pigmented	
Lactobacillus helveticus – 15	Colonies on MRS agar colonies white small, medium, large colonies, convex colonies with smooth edges, 1–3 mm in diameter, non pigmented	
Lactobacillus helveticus – 17	Colonies on MRS agar colonies white medium, large colonies, with uneven edges, are convex, 1–4 mm in diameter, non pigmented	
Lactobacillus helveticus -20	Colonies on MRS agar colonies white small, medium, large colonies, with uneven edges, are convex, 1–3 mm in diameter, non pigmented	
Lactobacillus helveticus – 22	Colonies on MRS agar colonies white small, medium, large colonies, with uneven edges, are convex, 1–3 mm in diameter, non pigmented	

All cultures were identified using MALDI BioTyper, with Score values ranging from 1.700 to 2.000 indicating a high degree of reliability. All 6 isolated strains were identified as *Lactobacillus helveticus*.

According to the literature data [15], lactobacilli have high antagonistic activity against pathogenic and opportunistic microorganisms. They are able to produce substances with antibiotic activity during their growth and development, the resulting antibiotic substance provides the dominance of lactobacteria and suppression of pathogenic microflora. Therefore, the use of lactic acid sticks with pronounced antagonistic activity in production has practical importance.

For probiotic purposes were used 6 isolated strains of lactic acid sticks in the study. It should be noted that most isolated strains showed good antagonistic activity. Table 3 and Figures 1–6 show the results of the study of antagonistic activity of isolated strains.

Table 3

Antagonistic activity of *Lactobacillus* isolates against different test-strains

Test strains	The diameters of the zones of growth inhibition (mm)					
	Lactobacillus helveticus – 15	*Lactobacillus helveticus – 22*	*Lactobacillus helveticus – 17*	*Lactobacillus helveticus – 20*	*Lactobacillus helveticus – 14*	*Lactobacillus helveticus – 13*
Staphylococcus aureus NCTC 12973/ ATCC® 29213™	14±1	16±1	23±1	34±2	11±1	13±1
Escherichia coli NCTC 12923/ ATCC® 8739™	0	0	0	19±1	29±2	13±1
Salmonella typhimurium NCTC 12023/ ATCC® 14028™	0	0	0	21±1	31±1	19±1
Klebsiella pneumonia NCTC 9633/ ATCC® 13883™	13±1	11±1	32±1	0	0	0
Pseudomonas aeruginosa NCTC 12903/ ATCC® 27853™	45±3	0	25±1	0	0	0
Streptococcus pyogenes NCTC 12696/ ATCC® 19615™	0	0	0	0	0	0
Candida albicans NCPF 3179/ ATCC® 10231™	0	0	0	0	0	0

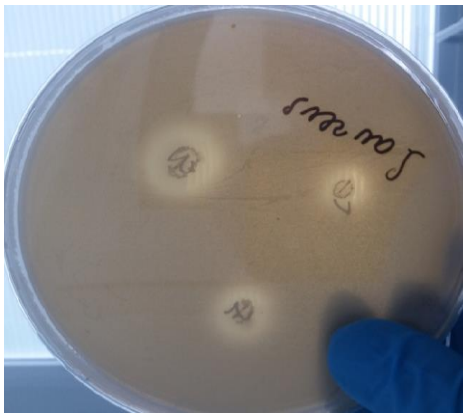


Figure 1. Antagonistic activity of *Lactobacillus* isolates against test-strains *Staphylococcus aureus* NCTC 12973/ ATCC® 29213™

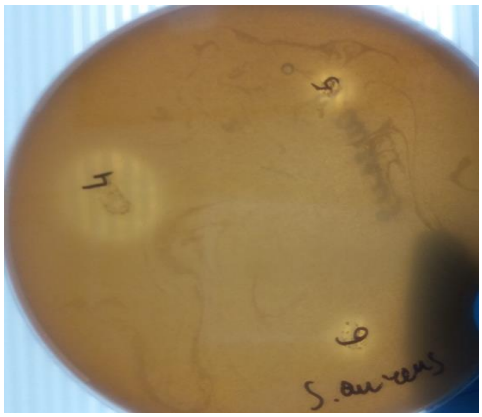


Figure 2. Antagonistic activity of *Lactobacillus* isolates against test-strains *Staphylococcus aureus* NCTC 12973/ ATCC® 29213™

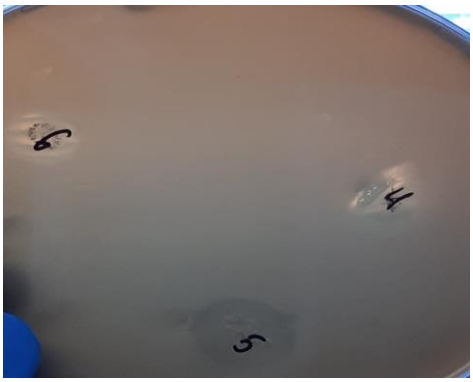


Figure 3. Antagonistic activity of *Lactobacillus* isolates against test-strains *Salmonella typhimurium* NCTC 12023/ ATCC® 14028™

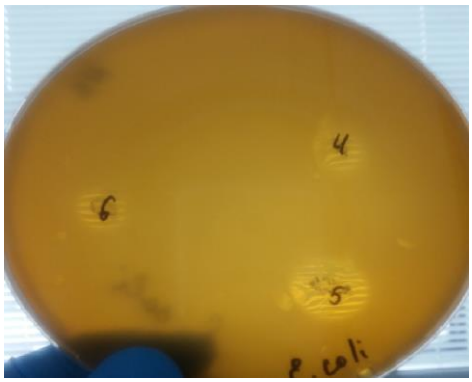


Figure 4. Antagonistic activity of *Lactobacillus* isolates against test-strains *Escherichia coli* NCTC 12923/ ATCC® 8739™

Figure 5. Antagonistic activity of *Lactobacillus* isolates against test-strains *Pseudomonas aeruginosa* NCTC 12903/ ATCC® 27853™

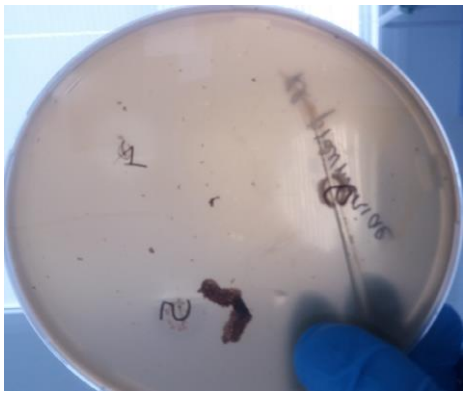


Figure 6. Antagonistic activity of *Lactobacillus* isolates against test-strains *Klebsiella pneumonia* NCTC 9633/ ATCC® 13883™

The studies revealed that only 6 strains of lactic acid sticks have low antagonistic activity to the test-strain:

– *Staphylococcus aureus* NCTC 12973/ATCC® 29213™ — *Lactobacillus helveticus – 15* — (14±1 mm), *Lactobacillus helveticus – 14* — (11±1 mm), *Lactobacillus helveticus – 13* — (13±1 mm).
– *Escherichia coli* NCTC 12923/ATCC® 8739™ — *Lactobacillus helveticus – 13* — (13±1 mm).
– *Klebsiella pneumonia* NCTC 9633/ATCC® 13883™ — *Lactobacillus helveticus – 15* — (13±1 mm), *Lactobacillus helveticus – 22* — (11±1 mm).

With respect to test strains, the following cultures have average antagonistic effect:

– *Staphylococcus aureus* NCTC 12973/ ATCC® 29213™ — *Lactobacillus helveticus – 22* — (16±1 mm).
– *Salmonella typhimurium* NCTC 12023/ ATCC® 14028™ — *Lactobacillus helveticus – 20* — (19±1 mm).
– *Escherichia coli* NCTC 12923/ ATCC® 8739™ — *Lactobacillus helveticus – 13* — (19±1 mm).

The following lactic acid stick cultures are active antagonists to test-strains:

– *Staphylococcus aureus* NCTC 12973/ ATCC® 29213™ — *Lactobacillus helveticus – 17* — (23±1 mm), *Lactobacillus helveticus – 20* — (34±2 mm).
– *Escherichia coli* NCTC 12923/ ATCC® 8739™ — *Lactobacillus helveticus – 14* — (29±2 mm).
– *Salmonella typhimurium* NCTC 12023/ ATCC® 14028™ — *Lactobacillus helveticus – 20* — (21±1 mm), *Lactobacillus helveticus – 14* — (31±1 mm).
– *Klebsiella pneumonia* NCTC 9633/ ATCC® 13883™ — *Lactobacillus helveticus – 17* — (32±1 mm).
– *Pseudomonas aeruginosa* NCTC 12903/ ATCC® 27853™ — *Lactobacillus helveticus – 15* — (45±3 mm), *Lactobacillus helveticus – 17* — (25±1 mm).

With respect to test strains, the following cultures were not antagonistic effect:

– *Escherichia coli* NCTC 12923/ ATCC® 8739™ — *Lactobacillus helveticus – 15, Lactobacillus helveticus – 22, Lactobacillus helveticus – 17.*
– *Salmonella typhimurium* NCTC 12023/ ATCC® 14028™ — *Lactobacillus helveticus – 15, Lactobacillus helveticus – 22, Lactobacillus helveticus – 17.*
– *Klebsiella pneumonia* NCTC 9633/ ATCC® 13883™ — *Lactobacillus helveticus – 20, Lactobacillus helveticus – 14, Lactobacillus helveticus – 13.*
– *Pseudomonas aeruginosa* NCTC 12903/ ATCC® 27853™ — *Lactobacillus helveticus – 22, Lactobacillus helveticus – 20, Lactobacillus helveticus – 14, Lactobacillus helveticus – 13.*

All 6 cultures of lactic acid sticks *Streptococcus pyogenes* NCTC 12696/ATCC ® 19615 ™*, Candida albicans* NCPF 3179/ATCC ® 10231 ™ — showed no antagonistic activity (no growth retardation zones).

Analyzing the experiment for antagonistic activity of isolated lactic acid stick strains, it can be concluded that *Lactobacillus helveticus – 17, Lactobacillus helveticus – 20, Lactobacillus helveticus – 14, Lactobacillus helveticus – 15* strains have high antagonistic activity.

Acid formation activity formation is a normalized indicator of biological activity of lactobacteria and accordingly a criterion for selection of lactobacteria strains with high-active probiotic properties. The results

obtained (Table 4) in the study show good acid-forming ability in most isolated lactic acid stick strains. Strains of lactic acid sticks, titrated acidity of which varies within 20–80 °T — are considered inactive, 90–110 °T — medium, and parameter 120 ° T and higher are considered highly active.

Table 4

Acid formation activity formation of *Lactobacillus* selected strains

Strains of the genus *Lactobacillus spp.*	Results, °T
Lactobacillus helveticus – 13	128,75
Lactobacillus helveticus – 14	131,33
Lactobacillus helveticus – 15	170,47
Lactobacillus helveticus – 17	183,86
Lactobacillus helveticus – 20	187,46
Lactobacillus helveticus – 22	100,94

The resistance of bacteria to antimicrobial preparation is a characteristic feature of a particular strain of the microorganism and this should be taken into account when selecting cultures, products and preparations with probiotic properties used in biotechnology. In this regard, we have conducted studies to determine the spectrum of antibiotic resistance of isolated strains of lactobacteria, to various most common antibiotics in medical practice. The obtained data on antibiotic sensitivity of lactobacteria are shown in Table 5.

Table 5

Antibiotic sensitivity of *Lactobacillus* selected strains

Antibiotics, μg/disc	The diameters of the zones of growth inhibition (mm)					
	Lactobacillus helveticus – 13	*Lactobacillus helveticus – 14*	*Lactobacillus helveticus – 15*	*Lactobacillus helveticus – 17*	*Lactobacillus helveticus – 20*	*Lactobacillus helveticus – 22*
Benzylpenicillin, 10	33±2	31±1	No zone of inhibition	No zone of inhibition	No zone of inhibition	37±1
Gentamycin, 10	30±1	34±2	21±1	20±1	19±1	35±1
Amoxyclav, 10	32±1	25±1	22±2	20±2	18±2	35±1
Tetracycline, 10	33±1	27+2	18±1	23±2	19±2	41±1
Levomycetin, 10	28±1	32±1	19±2	19±1	18±1	39±1
Cefuroxime, 30	32±2	25±1	16±2	No zone of inhibition	No zone of inhibition	15±2
Ciprofloxacin, 30	20±1	25±1	14±1	No zone of inhibition	No zone of inhibition	20±1
Clindamycin, 10	38±1	30±1	29±1	26±1	20±2	42±1
Colistin, 25	20±1	No zone of inhibition	No zone of inhibition	20±2	No zone of inhibition	30±2
Metronidazole, 5	No zone of inhibition	No zone of inhibition	No zone of inhibition	No zone of inhibition	No zone of inhibition	12±1

The study found that 5 strains had metronidazole resistant except *Lactobacillus helveticus – 22.*

Lactobacillus helveticus – 15, Lactobacillus helveticus – 17, Lactobacillus helveticus – 20 strains showed resistance to benzylpenicillin.

Growth retardation was not observed in *Lactobacillus helveticus – 17 strain, Lactobacillus helveticus* – 20 cefuroxime, ciprofloxacin.

The following strains of *Lactobacillus helveticus* – 14, *Lactobacillus* helveticus – 15, *Lactobacillus helveticus – 20* possess resistance to colistin.

Most strains of lactobacilli were sensitive to the following antibiotics: 3 strains to benzylpenicillin, levomicetin, cefuroxime, ciprofloxacin, colistin, gentamycin, 5 strains to amoxyclav, 4 strains to tetracycline, all 6 strains to clindamycin.

The following strains of lactic acid sticks showed considerable resistance: *Lactobacillus helveticus – 20* to gentamycin, amoxyclav, tetracycline, levomycetin. *Lactobacillus helveticus – 17* showed considerable resistance to levomitsetin. *Lactobacillus helveticus – 15* showed considerable resistance to tetracycline, levomycetin, cefuroxime, ciprofloxacin.

According to literary data [16], *in vitro* lactobacteria sensitivity tests are still poorly standardized. The evaluation of antibiotic sensitivity of these bacteria is extremely difficult because the size of the zones recommended for other bacteria are not applicable to them. To these reasons are added specific conditions of cultivation: enriched medium, complex composition of atmosphere, prolonged incubation.

Domestic microbiologists K.Kh. Almagambetov, I.S. Savitskaya with co-authors do not give the clear criteria for classification of strains of lactobacilli to sensitive or resistant [17]. Therefore, empirical concentrations in the antibiotic disc (μg/disc) that delayed the growth of at least one of the strains studied were selected as boundary minimum inhibitory concentrations (MIC).

Conclusions

1. Isolated strains of *Lactobacillus helveticus* are promising applicants and competitive strains for construction of the *Lactobacillus spp* consortium.

2. The use of modern microbiological methods allowed screening of isolated cultures and screening of biological active strains: *Lactobacillus helveticus – 17, Lactobacillus helveticus – 20, Lactobacillus helveticus – 14, Lactobacillus helveticus – 15,* which will form the basis of a consortium of microorganisms for wide use in our region. The relevance of the creation of new high-activity consortium based on strains of lactobacteria, extracted mainly from local sources, dictates the continuation of this study.

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Ж.Т. Амирханова, Р.Т. Бодеева, С.Б. Ахметова, А.Р. Кушугулова

Консорциум дизайны үшін Қарағанды облысынан бөлінген *Lactobacillus helveticus* штамдарының биологиялық қасиеттерін зерттеу

Әлемнің жетекші ғылыми орталықтарында адамның ішек жолдарының шартты-патогендік және патогенді микрофлорасының әртүрлі өкілдерінің антагонисі болып табылатын сүтқышқылды бактериялардың тірі дақылдары бар пробиотиктерді және дұрыс тамақтануға сүт өнімдерін алудың микробиология саласындағы зерттеулер ерекше өзектілікке ие. Пробиотикалық препараттар мен функционалдық сүт өнімдерінің тиімділігі бірінші кезекте олардың құрамына кіретін әртүрлі бактериялар штамдары түрлерінің қасиеттеріне байланысты. Осыған байланысты қазіргі уақытта пробиотикалық белсенділігі жоғары табиғи көздерден бөлінген сүтқышқылды штамдарды зерттеу бойынша басымдық артуда. Сүт өнімдері мен пробиотикалық препараттарға арналған бастапқы дақылдардың негізгі компоненттерінің бірі *Lactobacillus* туыстастығының бактериялары болып табылады. Мақалада S*taphylococcus aureus* NCTC 12973/ ATCC® 29213™, *Escherichia coli* NCTC 12923/ ATCC® 8739™, *Salmonella typhimurium* NCTC 12023/ ATCC® 14028™, *Pseudomonas aeruginosa* NCTC 12903/ ATCC® 27853™, *Klebsiella pneumonia* NCTC 9633/ ATCC® 13883™ тест-штамдарына антагонистік белсенділігі бар, Қарағанды облысында өндірілетін сүт өнімдерінен бөлініп алынған *Lactobacillus helveticus* штамының морфологиялық, дақылдық қасиеттері, қышқыл түзу қабілеті, антибиотиктерге сезімталдығының зерттеулері ұсынылған. Қазіргі заманғы микробиологиялық зерттеу әдістері бөлінген дақылдарға скрининг жүргізуге және биологиялық белсенді штамдарды іріктеуге мүмкіндік берді: *Lactobacillus helveticus – 17, Lactobacillus helveticus – 20, Lactobacillus helveticus – 14, Lactobacillus helveticus – 15*. Алынған нәтижелер бойынша, *Lactobacillus helveticus* штамдары *Lactobacillus spp*. консорциумына үміткерлер болып табылады және бұл штамдар бәсекеге қабілетті деп айтуға мүмкіндік беріп отыр.

Кілт сөздер: дақылдандыру, штамм, Грам әдісімен бояу, *Lactobacillus helveticus*, антагонистік белсенділік, қышқылтүзу белсенділігі, антибиотикке сезімталдық.

Ж.Т. Амирханова, Р.Т. Бодеева, С.Б. Ахметова, А.Р. Кушугулова

Изучение биологических свойств штаммов *Lactobacillus helveticus,* выделенных в Карагандинской области для дизайна консорциума

В ведущих научных центрах мира особую актуальность приобретают исследования в области микробиологии получения молочных продуктов здорового питания и пробиотиков, содержащих живые культуры молочнокислых бактерий, являющихся антагонистами различных представителей условно-патогенной и патогенной микрофлоры кишечного тракта человека. Эффективность пробиотических препаратов и продуктов молочного функционального питания, в первую очередь, зависит от свойств, входящих в их состав видов различных штаммов бактерий. В связи с этим в настоящее время приоритет отдается изучению молочнокислых штаммов, выделенных из природных источников, обладающих высокой пробиотической активностью. Одним из основных компонентов стартерных культур для молочных продуктов и пробиотических препаратов чаще всего являются бактерии рода *Lactobacillus*. В статье представлены морфологические, культуральные свойства, кислотообразующая способность, антибиотикочувствительность *Lactobacillus helveticus,* выделенных в Карагандинской области, обладающих антагонистической активностью по отношению к тест-штаммам: S*taphylococcus aureus* NCTC 12973/ ATCC® 29213™, *Escherichia coli NCTC* 12923/ ATCC® 8739™, *Salmonella typhimurium* NCTC 12023/ ATCC® 14028™, *Pseudomonas aeruginosa* NCTC 12903/ ATCC® 27853™, *Klebsiella pneumonia* NCTC 9633/ ATCC® 13883™. Использование современных микробиологических методов позволило провести скрининг выделенных культур и отселектировать биологические активные штаммы: *Lactobacillus helveticus – 17, Lactobacillus helveticus – 20, Lactobacillus helveticus – 14, Lactobacillus helveticus – 15*. Согласно полученным результатам, штаммы *Lactobacillus helveticus* являются перспективными претендентами на консорциум *Lactobacillus spp*. и позволяют судить о конкурентоспособности данных штаммов.

Ключевые слова: культивирование, штамм, окраска по Граму, *Lactobacillus helveticus*, антагонистическая активность, кислотообразующая активность, антибиотикочувствительность.

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Assessment of water-retaining capacity of wheat seedlings after exposure to laser radiation

The article presented the results of assessing the effect of hydration and water-holding capacity of soft wheat seedlings after preliminary treatment of seeds with a laser with a wavelength of 632,8 nm. The processing timer anged from 15 seconds to 15 minutes. The watering of raw seedlings during drying and air-dry weight was higher than the control values. Thus, the water content of 3-week-old wheat seedlings in all experimental variants exceeded the control by 12.5–45.2 %. The weight of the experimental seedlings after 2 hours of drying exceeded the control value by 23.5–159.4 %, after 4 hours — by 5.6–74.5 %. The dry weight of the seedlings turned out to be 6.7–44.4 % higher than the control in the experimental variants. The water-holding capacity of wheat seedlings according to the experimental variants turned out to be approximately at the control level or higher than the control values. These parameters indicated a positive effect of laser processing of a certain duration on the increase in drought resistance. The best indicators of seedling water content and water-holding capacity were noted for pre-sowing treatments with laserir radiation — 1 minute, 2 minutes, 2 minutes 30 seconds, 4 minutes, 10 minutes.

Keywords: seed material, seedlings, wheat, watercontent, water-holding capacity, laserir radiation, experimental options

Introduction

Water is an internal environment where all the processes of vital activity take place actively; it is a transport link between various structures of a living organism.

The territory of Kazakhstan differs in the amount of precipitation, and most of it is in the arid climate zone [1]. The climate of Central Kazakhstan (Karaganda region) is characterized by sharp continental, arid in the summer months and a small amount of precipitation from 180 to 310 mm [2]. The greatest harm is caused by drought in the spring and summer, during which there is an active growth of crops and the formation of generative organs [3].

In natural conditions, a favorable combination of soil-climatic and agrometeorological factors is extremely rarely achieved throughout the growing season. Often crop failures in the Central and the Northern Kazakhstan are caused by frequent droughts in the first half of summer [4], which determines the need to find ways to increase drought tolerance of agricultural plants.

The resistance of plants to drought is determined by a number of factors, the most important of which is the water regime of plants [5–7], that is, the ability of the plant's aboveground organs to retain water. As noted by a number of authors [8–12], the rate of water return is often used as an indicator of drought tolerance of plants; therefore, plants with high water retention capacity are highly resistant to adverse environmental conditions. Therefore, when establishing the resistance of plants to drought, this indicator is used as a diagnostic sign.

The aim of research is to study the dynamics of changes in water content and water holding capacity of wheat seedlings irradiated with different doses of coherent laser radiation.

Methodology

The object of the study was soft wheat seeds (*Triticale aestivum* L.) of "Karaganda-29" variety, obtained in 2018 from the Karaganda Research Institute of Plant Growing and Breeding of the Ministry of Agriculture of the Republic of Kazakhstan. Seed samples were irradiated with a helium-neon laser; a wavelength of 650 nm was used, the irradiation time varied from 15 seconds to 15 minutes. The control was seeds that were not exposed to laser irradiation.

All irradiated seed samples were planted in boxes with standard soil for growing seedlings. Each version of the experiment was in 6 replicates, in each repetition — 50 pieces of seeds. After 3 weeks of cultivation in closed ground, plant seedlings were dug up, washed from the ground and weighed on wet weight.

The water content of plants was estimated by the ratio of the wet and dry weight of the seedlings. Water retention capacity was determined after 2 and 4 hours of wilting (in % of fresh weight) according to the method of M.D. Kushnirenko [13] and Yu.V. Makarova [14].

In the final phase, the plants were placed in filter bags and dried in weights to constant weight at 100 °C. Mass fraction of moisture (%) was calculated by the formula:

$$100\ \% - A,$$

where, A is the mass of absolutely dry matter, %.

$$A = (m - m_1) \times 100\ \% / m_2,$$

where, m is the mass of the seedling drying, g; m_1 is the mass of the seedling after drying, g; m_2 is the mass if fresh seedling weight, g.

The water-holding ability of seedlings (%) was estimated by the formula:

$$B = \frac{C - D}{E} * 100\ \%,$$

where E is the absolute water content; C is the raw mass of seedlings before drying; D is the dry mass of seedlings.

Statistical processing of the results was carried out according to the method of N.L. Udolskaya [15].

Results and discussion

The results of the experiments showed that the content of free water and the water-holding ability of wheat seedlings differed in the experiment variants (Table 1).

Table 1

Indicators of the water-holding ability of wheat seedlings after laser irradiation of various durations

Experience options	Wetweight, g	Weight after 2 hours of drying, g	Weight after 4 hours of drying, g	Air-dry weight of seedlings, g
Control	0.239±0.001	0.170±0.001	0.110±0.001	0.045±0.001
15 seconds	0.290±0.002	0.190±0.002	0.135±0.002	0.060±0.002
30 seconds	0.299±0.002	0.207±0.003	0.132±0.002	0.055±0.002
1 minute	0.322±0.003	0.252±0.002	0.168±0.002	0.067±0.002
1 min 30 sec	0.243±0.002	0.157±0.001	0.118±0.002	0.048±0.002
2 minutes	0.333±0.001	0.270±0.001	0.192±0.002	0.064±0.001
2 min 30 sec	0.306±0.002	0.225±0.002	0.165±0.002	0.058±0.002
3 minutes	0.340±0.003	0.251±0.002	0.182±0.002	0.064±0.002
3 min 30 sec	0.269±0.002	0.174±0.001	0.116±0.002	0.050±0.001
4 minutes	0.272±0.001	0.206±0.002	0.152±0.001	0.056±0.002
4 min 30 sec	0.369±0.003	0.271±0.001	0.186±0.002	0.067±0.002
5 minutes	0.309±0.003	0.219±0.001	0.151±0.002	0.059±0.002
10 minutes	0.339±0.001	0.241±0.001	0.176±0.002	0.065±0.001
15 minutes	0.347±0.002	0.237±0.001	0.165±0.001	0.060±0.002

An analysis of the results shows that the wet weight of 3-week-old wheat seedlings was higher in all variants of experiments with laser irradiation, exceeding the control values by at least 12.5 % (3 minutes 30 seconds) and a maximum of 45.2 % (15 minutes) (Fig. 1). Similar results were obtained when drying after 2 (with the exception of the experiment with a processing time of 1 minute 30 seconds) and 4 hours. After 2 hours, the weight of the seedlings exceeded the control values by 0.04–0.1 g, that is, from 23.5 to 159.4 %. The maximum excess values are noted in the processing options 1 minute, 2 minutes, 4 minutes 30 seconds, 10 minutes.

After drying for 4 hours, all experimental options exceeded the control, showed from 0.06 to 0.082 g, or 5.6 to 74.5 %. At this stage of the experiment, the maximum showed the weight of the seedlings marked for variants with a processing time of 1 minute, 2 minutes, 3 minutes, 4 minutes, 4 minutes 30 seconds, 5 and 10 minutes.

The dry weight of plants in all cases exceeded the control; the difference was 0.003–0.02 g or 6.7–44.4 % (Fig. 2). That is, we can observe an increase in plant water content after laser treatment, which is a sign of an increase in the ability of plants to tolerate drought [16, 17].

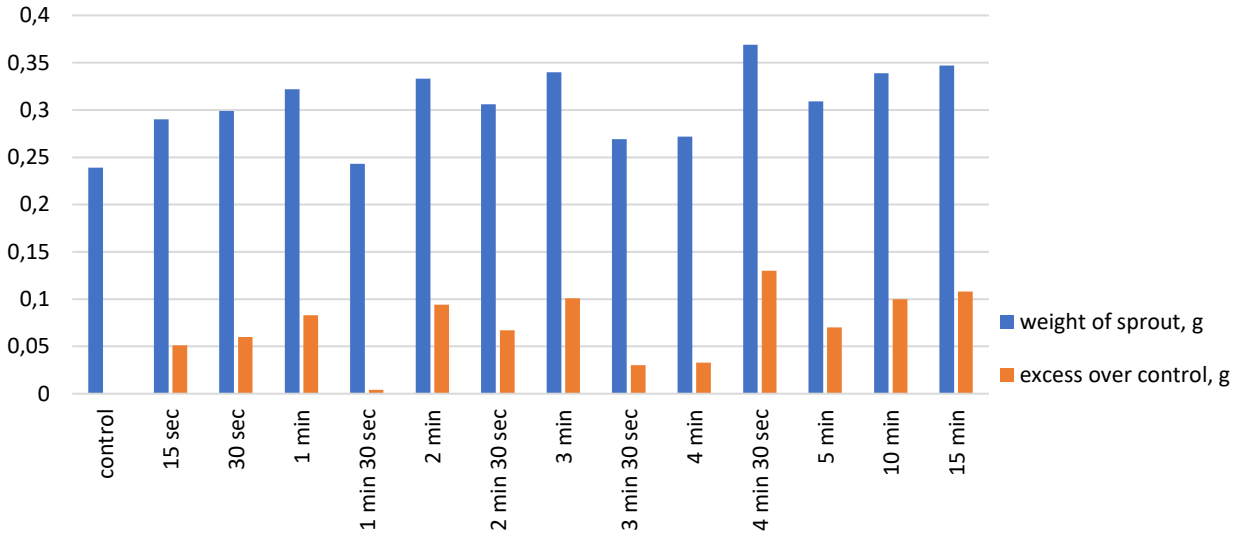

Figure 1. Indicators of fresh weight of seedlings and data of excess over control for experimental options

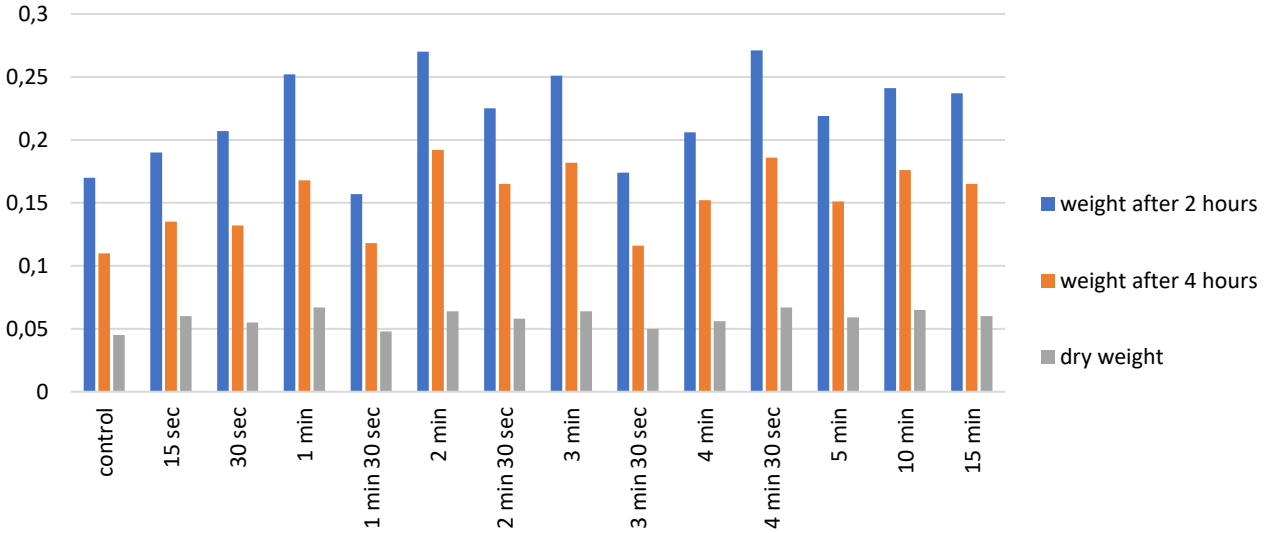

Figure 2. Indicators of the weight of seedlings during drying and dry weight according to the options of experience

We have determined the water holding capacity of seedlings. Significant differences were expressed in terms of hydration (water content) of seedlings before drying in the range of 43 and 58 %. A more significant decrease in humidity was observed during irradiation in the indicated interval: 3 min 30 seconds. In this irradiation interval, the water holding capacity decreased by 3 % (from 33 % to 30 %) compared with the control. The second peak of the water holding capacity of the seeds was observed in the intervals of 2, 3, 4 minutes. At these doses of laser radiation, the values increased by 10–18 % (from 30 to 40–48 %) (Table 2).

Compared with the control, the water-holding ability increased by 10–15 % in samples that were under the influence of laser radiation for 2, 3, and 4 minutes. The indicated irradiation time intervals are the most optimal for water retention in wheat seedlings.

However, laser radiation of seeds not all variants of the experiment led to an increase in water retention capacity. So, samples those were irradiated for 15, 30 and 210 seconds showed values below the control. For example, wheat seedlings in the control variant had indicators of water holding capacity of 33 %, and in some variants this value slightly decreased by 2–3 % (Fig. 3).

Table 2

Water content and water holding capacity of wheat seedlings at different laser irradiation times

Experience Options	Water content, %			Waterholding capacity, %
	In 2 hours	In 4hours	Dry seedlings	
Control	71	46	18	33
15 seconds	66	47	21	32
30 seconds	69	44	18	31
1 minute	78	52	21	40
1 min 30 sec	65	49	20	36
2 minutes	81	58	19	48
2 min 30 sec	74	54	19	43
3 minutes	74	54	19	43
3 min 30 sec	65	43	19	30
4 minutes	76	56	21	44
4 min 30 sec	73	50	18	39
5 minutes	71	49	19	37
10 minutes	71	52	19	41
15 minutes	68	48	17	37

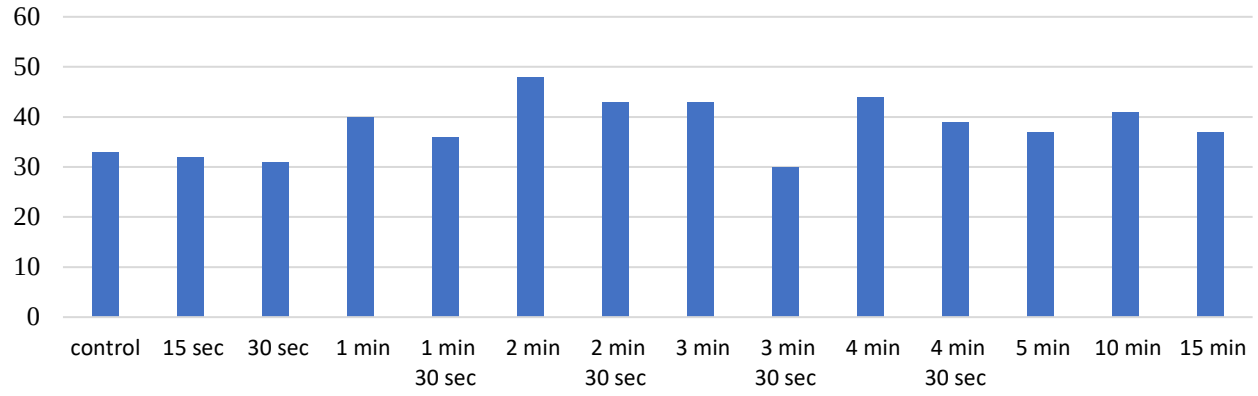

Figure 3. Indicators of the water-holding ability of wheat seedlings according to the experimental variants after laser irradiation

In general, the results show that most variants of pre-sowing treatment of seeds with a coherent laser lead to an increase in the water content of seedlings and an increase in water retention capacity.

Conclusion

Processing of seeds of agricultural plants leads to an increase in germination and activates the growth of seedlings. We carried out pre-sowing treatment of seeds with a laser with a wavelength of 650 nm and the duration of 15 seconds to 15 minutes. The irrigation of 3-week-old seedlings obtained in closed ground in all experimental variants using laser treatment turned out to be higher than the control values. The excess over control was 12.5–45.2 %. The loss of moisture during drying took place approximately the same in all variants of the experiment. The weight of the seedlings after 2 and 4 hours of drying exceeded the control by 23.5–159.4 % and 5.6–74.5 %, respectively.

The dry weight of the seedlings turned out to be 6.7–44.4 % higher than the control in the experimental variants.

The water-holding ability of wheat seedlings according to the experimental variants turned out to be approximately at the control level or higher than the control values. The best indicators of seedling water content and water-holding ability were noted for pre-sowing treatments with laser irradiation — 1 minute, 2 minutes, 2 minutes 30 seconds, 4 minutes, 10 minutes.

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Д.М. Әменова, Д.В. Агеев, М.Ю. Ишмуратова, А.К. Зейнидинов

Лазер сәулесін қолданғаннан кейін бидай дақылы өскіндерінің суды ұстап тұру қабілетін бағалау

Мақалада толқын ұзындығы 632,8 нм лазермен алдын ала өңделген жұмсақ бидай өскіндерінің суды ұстап тұру қабілеті мен суландыруға әсер етуін бағалау келтірілген. Өңдеу уақыты 15 секундтан 15 минутқа дейінгі аралықты қамтиды. Кептіру кезінде ылғал өскіндердің сулануы мен олардың ауадағы құрғақ массалары бақылау үлгісімен салыстырғанда жоғарырақ болды. Сонымен, бидайдың 3 апталық өскіндерінің сулануы бақылаумен салыстырғанда 12,5–45,2 %-ға артты. Тәжірибелік массалардың 2 сағат кептірілгеннен кейінгі мәндері бақылаумен салыстырғанда 23,5–159,4 %-ға, ал 4 сағаттан кейін 5,6–74,5 %-ға жоғары болды. Тәжірибедегі өскіндердің құрғақ массалары бақылаумен салыстырғанда 6,7–44,4 %-ға артты. Бидай өскіндердің суды ұстап тұру қабілеті бақылаумен тең және жоғары мәндерге ие болды. Берілген көрсеткіштер белгілі бір ұзақтықта лазер сәулесімен алдын ала өңдеу шөлге тұрақтылық көрсеткіштерін арттырып, оң әсер береді. Өскіндердің сулануы мен суды ұстап тұруға қабілеттілігінің ең жақсы көрсеткіштері лазер сәулесімен атқыланған мына нұсқаларда: 1 минут, 2 минут, 2 минут 30 секунд, 4 минут, 10 минутта байқалды.

Кілт сөздер: тұқым материалы, өскіндер, бидай, сулану, суды ұстап тұру қабілеті, лазер сәулесі, тәжірибе нұсқалары.

Д.М. Аменова, Д.В. Агеев, М.Ю. Ишмуратова, А.К. Зейнидинов

Оценка водоудерживающей способности проростков пшеницы после применения лазерного облучения

В статье приведены оценки влияния оводненности и водоудерживающей способности проростков пшеницы мягкой после предварительной обработки семян лазером с длиной волны 632,8 нм. Длительность обработки составляла от 15 сек до 15 мин. Оводненность сырых проростков в процессе высушивания и на воздушно-сухой вес оказалась выше контрольных значений. Так, оводненность 3-недельных проростков пшеницы по всем вариантам опыта превысила контроль на 12,5–45,2 %. Вес опытных проростков после 2-х ч высушивания превышал значения контроля на 23,5–159,4 %, после 4-х ч — на 5,6–74,5 %. Сухой вес проростков оказался в опытных вариантах выше контроля на 6,7–44,4 %. Водоудерживающая способность проростков пшеницы по вариантам опыта оказалась примерно на уровне контроля или выше контрольных значений. Данные показатели свидетельствуют о положительном влиянии лазерной обработки определенной длительности на повышение показателей устойчивости к засухе. Лучшие показатели оводненности проростков и водоудерживающей способности отмечены при вариантах предпосевной обработки лазерным облучением — 1 мин, 2 мин, 2 мин 30 сек, 4 мин, 10 мин.

Ключевые слова: семенной материал, проростки, пшеница, оводненность, водоудерживающая способность, лазерное облучение, варианты опыта.

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Identification of new strains of lactic acid bacteria from south region of Kazakhstan

The article describes the process of isolation and identification of lactic acid microorganisms derived from traditional homemade dairy products of the Southern region of Kazakhstan. During the study, 10 samples were selected. Of these, 5 separate isolates were selected: 2 koumiss isolates (Al-2, Al-3) and 3 ayran isolates (Al-1, Al-4, Al-5). A microscopic observation of koumiss isolates showed that they were cocci, isolated and collected in chains, and ayran isolates were large long rods. Spores were not formed. Bacteria were gram-positive facultative anaerobes. Colonies were convex with a solid edge, opaque and not pigmented. Optimum growth temperature was 37 °C. Identification was done by the MALDI-TOF method of mass spectrometry using the Microflex device based on the Maldi Biotyper database. Mass spectra were compared using Biotyper 3.0 RTC software. Based on the results, the Score value of the analyzed strains ranged from 2.096 to 2.449. As a result, it was found that isolates Al-2, Al-3 extracted from koumiss belong to the species *Lactococcus lactis* and isolates Al-1, Al-4, Al-5 extracted from ayran to *Lactobacillus plantarum*. The data obtained by us indicate that the results of microbiological identification are consistent. Antagonistic activity of cultures was studied by 2 test strains (*S. aureus* and *E. Coli*) using agar block method. The results showed that the lysis zone around the wells with *Staphylococcus aureus* culture was in the range from 12.00±1 mm to 18.00±1 mm, and *Escherichia coli* in the range from 13.00±1 mm to 17.00±1 mm. The isolates have an inhibitory effect on the indicator test strains: *Staphylococcus aureus* and *Escherichia coli*.

Keywords: lactic acid products, lactic acid microorganisms, isolates, strain, *Lactobacillus spp*., *Lactococcus spp*., antagonistic activity, mass spectrometry.

Introduction

Preservation of biodiversity is a pressing challenge of modern time. This is reflected in the International Convention on Biological Diversity [1], which has been ratified by the Republic of Kazakhstan. The conservation and sustainable use of biological diversity is crucial to meet the food and healthcare needs, as well as other needs of the world's growing population. Thus, access to and sharing of both genetic resources and technologies are essential to handle these challenges.

Bio resource centers based on collections of microorganisms, viruses, cell cultures of plant and animal tissues, are the basis for the development of biotechnology. Interest in the collections has particularly risen since the second half of XX century. When, with the establishment of new biotechnological production facilities, the need for strains of microorganisms with certain properties has sharply increased, which has intensified work on obtaining highly active producers, a deeper study of supported microbial collections and the creation of data banks [2–4].

The effective use of modern biotechnologies in health care, agriculture, pharmacy, processing and food industry is an important prerequisite for the development and solution of problems in these sectors, as well as for environmental protection [5].

The issues of production and consumption of milk and dairy products are becoming more urgent and increasingly dependent on general trends of the world food market development.

Recently, there is an increased interest in studying lactic acid bacteria. This is largely due to the rapid development of the dairy industry worldwide, production of new fermented milk products, and the search for new strains of lactic acid bacteria suitable for use in fermentation starter.

Dairy products are an essential component of the human diet. They account for satisfaction of up to 20 % of human protein and up to 30 % of fat intake [6].

Today, from a wide range of food products, the customer often chooses those that have additional properties and advantages, such as naturality, health benefits, unusual taste, convenience and others.

Many international manufacturers strive to follow these trends and offer new solutions for people who want to improve their health. Products that help reduce fat tissue and contain pro-biotic ingredients, vitamins, minerals, dietary fiber, fatty acids, etc. are becoming increasingly popular.

Thus, the variety of dairy products is due to the use of bacterial ferments, the composition of which is represented by different types of lactic acid bacteria. The specific taste, consistency and several other properties of milk products depend on the strains that make up the bacterial starters.

The purpose of this project was to isolate and identify lactic acid bacteria from various traditional dairy products of the Southern region of Kazakhstan.

Materials and research methods

Samples of home-made dairy products (ayran, koumiss) selected in the southern region of Kazakhstan were used as study materials.

In order to obtain the accumulation culture of lactic acid strains, the following nutrient medium was used: skimmed milk powder (87 g/L); yeast autolysate (3 ml). Cultivation was carried out at 37 °C, during 16–24 hours.

Pure cultures were isolated by tenfold dilution followed by inoculation on Petri dishes with MRS agar medium. Grown isolated colonies were transferred with a loop to slant agar in tubes and cultivated at 37 °C for 48 hours. Culture purity was observed for absence of extraneous growth in the beef-extract broth.

The cultures of isolates were visually observed using a phase contrast microscope.

Mass spectrometric identification of lactic acid bacteria was performed by the MALDI-TOF mass spectrometry method using the Microflex device based on the Maldi Biotyper database (Bruker Daltonics, Germany).

Antagonistic activity of cultures was studied by 2 test strains: *Staphylococcus aureus* and *Escherichia coli* using agar block method [7].

Results and discussion

During the study 10 samples were taken from traditional homemade dairy products. Of these, 5 separate isolates were obtained, including: 2 koumiss isolates (Al-2, Al-3) and 3 ayran isolates (Al-1, Al-4, Al-5).

Identification of the isolates was made on the basis of cultural, morphological and physiological features using "Bergey's manual". A microscopic observation of koumiss isolates showed that they were cocci, isolated and collected in chains, and ayran isolates were large long rods. Spores were not formed. Bacteria were gram-positive facultative anaerobes. Colonies were convex with a solid edge, opaque and not pigmented. Optimum growth temperature was 37 °C [8].

As a result of the study of cultural and morphological properties, the isolated bacteria were identified as representatives of the genus *Lactobacillus spp*. and *Lactococcus spp*.

Proteomics using matrix-assisted laser desorption/ionization and time-of-flight mass-spectrometry (MALDI-TOF) has been rapidly developing in microbiology in recent years. The basic principle of MALDI-TOF technology is the use of a matrix solution which crystallizes from the sample and matrix substrate after evaporation of the solvent. The formation of the matrix-sample will absorb energy when exposed to laser and transfer ion charge from matrix to sample. When charged samples enter the vacuum tube and the accelerating electric field of the system, fragments of charged samples will be separated based on their mass-to-charge ratio and the flight detector will analyze this separation based on mass and charge and generate what is known as mass spectra. Using software and algorithmic analysis, the mass spectra for a sample are compared with the mass spectra for known species contained in the system database [9].

Identification by the MALDI-TOF method of mass spectrometry has been carried out using the Microflex instrument based on the Maldi Biotyper database (Bruker Daltonics, Germany). As a matrix, a α-cyano-4-hydroxycoric acid in 50 % acetonitrile with addition of 2.5 % trifluoroacetic acid was used. For identification, microbial colonies were used after primary inoculation. The colony was applied to a metal target, covered with a matrix solution, and after drying, microorganisms were identified in a mass spectrometer by ribosomal protein spectra. The results are presented in Table 1.

The mass spectra were compared using the Biotyper 3.0 RTC program (Bruker Daltonics, Germany). The degree of identification reliability was assessed using the obtained Score values, comparing the spectra data from the Biotyper 3.0 reference database. Cases with Score <1.7 were considered as unreliable and were not considered as cases of successful determination of taxonomic properties of an isolate [10].

The results presented in Table 1 show that the Score value of the analyzed strains varies from 2.096 to 2.449.

Table 1

Mass spectrometric identification results

№	Sample	Homologous bacteria	Score value	Quality	Number from NCBI database
1	AL-1	*Lactobacillus plantarum* DSM 2601 DSM	2.19	++	1590
2	AL-2	*Lactococcus lactis ssp. lactis* DSM 20661 DSM	2.449	+++	1360
3	AL-3	*Lactococcus lactis* DSM 4366 DSM	2.096	++	1358
4	AL-4	*Lactobacillus plantarum* DSM 2601 DSM	2.152	++	1590
5	AL-5	*Lactobacillus plantarum* DSM 1055 DSM	2.206	++	1590

Notes. 1 "+" identification of the genus (1.700...1.999); 2 "++" genus identification, identification of probable species (2.000...2.999); 3 "+++" species identification (2.300...3.000).

As a result, it was found that isolates Al-2, Al-3 derived from koumiss belong to the species *Lactococcus lactis* and isolates Al-1, Al-4, Al-5 resulted from ayran to *Lactobacillus plantarum*. The data we have obtained indicate that the results of microbiological identification are consistent with the following.

When creating products based on several strains, an attention should be given to studying antagonistic properties. The antagonism of Lactic acid bacteria (LAB) is caused by the production of lactic acid, which has a certain bactericidal effect and, besides, causes the pH of the medium to drop to values unfavorable for many microorganisms [11].

Antagonistic activity of cultures was studied by 2 test strains: *S. aureus* and *E. coli* by agar blocks method. The test strains were obtained from the collection of microorganisms of the Branch of RSE "National Center of Biotechnology" of the Academy of Sciences of the Republic of Kazakhstan in Stepnogorsk.

Inoculants of *Staphylococcus aureus* and *Escherichia coli* cultures were mixed with heated and cooled to 50 °C agar and poured into Petri dishes. The dishes were cultivated at 37 °C for 20 min. Symmetrically arranged discs with a diameter of 10 mm were cut out of the agar plates obtained by the deep method, and a suspension of selected cultures was introduced into the wells. After the suspension was introduced into wells, the tested plates were incubated at 37 °C for 24 hours. Antagonistic activity was judged by the absence of zone of test strains growth around the colony of the tested isolate. The antagonistic activity was differentiated by 4 degrees: zero in the zone of no growth up to 1.0 mm, low 1.1 4.9 mm, average 5.0 8.9 mm, high 9.0 mm and more (Table 2).

Table 2

Antagonistic activity assessment results

Sample	*Staphylococcus aureus*	*Escherichia coli*
	Zone diameter, mm	
Lactobacillus plantarum AL-1	14,0±1	13,0±1
Lactococcus lactis AL-2	13,0±1	12,0±1
Lactococcus lactis AL-3	12,0±1	13,0±1
Lactobacillus plantarum AL-4	15,0±1	14,0±1
Lactobacillus plantarum AL-5	18,0±1	17,0±1

The results showed that the lysis zone around the wells with *Staphylococcus aureus* culture is in the range from 12.00±1 mm to 18.00±1 mm, and *Escherichia coli* is in the range from 13.00±1 mm to 17.00±1 mm. The results of the study of antagonistic activity showed that the studied isolates have an inhibitory effect on the indicator test strains: *Staphylococcus aureus* and *Escherichia coli*.

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Оңтүстік Қазақстан өңірінен алынған сүтқышқылды бактериялардың жаңа штамдарын идентификациялау

Мақалада Оңтүстік Қазақстан өңірінің түрлі дәстүрлі сүтқышқылды өнімдерінен бөлініп алынған сүтқышқылды микроорганизмдердің бөлінуі мен идентификациясы сипатталған. Жұмысты орындау барысында 10 үлгі іріктеліпп алынған. Олардан 5 жекеленген изоляттар бөлінді, оның ішінде: 2 изолят (Al-2, Al-3) қымыздан және 3 изолят (Al-1, Al-4, Al-5) айраннан. Қымыз изоляттарын микроскопиялық бақылау нәтижесінде, олардың бір-бірімен жеке және тізбектеп орналастырылған коктар, ал айраннан жасалған изоляттар үлкен ұзын таяқшалар екенін көрсетті. Спора пайда болған жоқ. Факультативті анаэробтар. Грамоң. Колониялар тұтас шеті дөңес, мөлдір емес және пигменттелмеген. Оңтайлы өсу температурасы 37 °С. Maldi-TOF масс-спектрометрия әдісімен, Maldi Biotyper деректер базасы негізінде Microflex құралын қолдана отырып, идентификациялау жүргізілген. Масс-спектрлерді салыстыру Biotyper 3.0 RTC бағдарламасының көмегімен жүргізілді. Нәтиже бойынша талдау жасалған штамдардың Score мәні 2.096-ден 2.449-ға дейін өзгерді. Жүргізілген талдаулар нәтижесінде, қымыздан алынған Al-2, Al-3 изоляттар *Lactococcus lactis,* ал айраннан алынған Al-1, Al-4, Al-5 изоляттары *Lactobacillus plantarum* түріне жататындығы анықталды. Алынған мәліметтер микробиологиялық сәйкестендіру нәтижелерінің сәйкестігін растады. Өсіріндінің антагонистік белсенділігі *S. aureus* және *E. coli* тест-штаммдарына блокты агар әдісімен зерттелген. Нәтижесінде, *S. aureus* өсіріндісімен шұңқырдың айналасындағы лизис аймағы 11,00±1 мм-ден 18,00±1 мм-ге дейінгі диапазонда, ал *E. coli* 11,00±1 мм-ден 17,00±1 мм-ге дейінгі диапазонда екенін көрсетті. Зерттелінген изоляттар *Staphylococcus aureus* және *Escherichia coli* индикаторлық тест-штамдарға қатысты тежеуші әсерге ие екенін көрсетті.

Кілт сөздер: сүтқышқылды өнімдер, сүтқышқылды микроағзалар, изоляттар, штамм, *Lactobacillus spp.*, *Lactococcus spp.*, антагонистік белсенділік, масс-спектрометрия.

Л.Ж. Байрон, А.О. Исенова, Н.К. Жаппар, В.М. Шайхутдинов

Идентификация новых штаммов молочнокислых бактерий из Южного региона Казахстана

В статье описан процесс выделения и идентификации молочнокислых микроорганизмов, полученных из традиционных молочнокислых продуктов домашнего приготовления Южного региона Казахстана. В ходе выполнения работы было отобрано 10 образцов. Из них было выделено 5 отдельных изолята, в том числе 2 изолята из кумыса (Al-2, Al-3) и 3 изолята из айрана (Al-1, Al-4, Al-5). Микроскопическое наблюдение изолятов из кумыса показало, что они представляют собой кокки, расположенные единично и собранные в цепочки, а изоляты из айрана — крупные длинные палочки. Спор не

образовывали. Факультативные анаэробы. Грамположительные. Колонии выпуклые, с цельным краем, непрозрачные и не пигментированы. Оптимальная температура роста — 37 °С. Проведена идентификация методом MALDI-TOF масс-спектрометрии с применением прибора Microflex на основе базы данных Maldi Biotyper. Сравнение масс-спектров проводилось с помощью программы Biotyper 3.0 RTC. По результатам значения Score проанализированных штаммов варьируются от 2,096 до 2,449. Было установлено, что изоляты Al-2, Al-3, выделенные из кумыса, относятся к виду *Lactococcus lactis*, а изоляты Al-1, Al-4, Al-5, выделенные из айрана, — к *Lactobacillus plantarum.* Полученные данные свидетельствуют о соответствии результатов микробиологической идентификации. Антагонистическую активность культур исследовали к 2 тест-штаммам: *S. aureus* и *E. coli* методом агаровых блоков. Результаты показали, что зона лизиса вокруг лунок с культурой *Staphylococcus aureus* находится в диапазоне от 12,00±1 мм до18,00±1 мм, а *Escherichia coli* — в пределах от 13,00±1 мм до 17,00±1 мм. Исследуемые изоляты обладают ингибирующим действием в отношении индикаторных тест-штаммов: *Staphylococcus aureus* и *Escherichia coli*.

Ключевые слова: молочнокислые продукты, молочнокислые микроорганизмы, изоляты, штамм, *Lactobacillus spp., Lactococcus spp.*, антагонистическая активность, масс-спектрометрия.

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Разработка протокола массового получения каллусных культур фасоли казахстанской и зарубежной селекции

Современным направлением для создания экологически безопасных комплексных биопрепаратов является их получение на основе культуры клеток и тканей растений. Во многом продуктивность культивируемых клеток значительно превышает продуктивность растений для выделения биологически активных веществ, ценных для медицины, сельского хозяйства, пищевой промышленности. Цель данного исследования — разработка протокола массового получения каллусных культур фасоли, являющихся альтернативным источником получения лектинов, используемых в производстве биопрепаратов для сельского хозяйства. Оптимизированы условия введения в культуру *in vitro* шести сортообразцов фасоли казахстанской и зарубежной селекции, установлен оптимальный состав питательных сред для индукции каллусогенеза, определены сортообразцы, обладающие высокой каллусообразующей способностью. Установлена зависимость частоты каллусогенеза от минерального состава питательной среды и типа экспланта. Наибольшая частота каллусообразования наблюдалась в среде УМ (80–100 %), а наиболее подходящим эксплантом для индукции каллуса является эпикотиль. Показана зависимость частоты формирования морфогенного каллуса в зависимости от типа и концентрации фитогормонов. Образование морфогенных каллусов наблюдалось только в присутствии 2,4-Д. Максимальный выход морфогенного каллуса отмечен при концентрации 2,4-Д 2 мг/л. Выявлены перспективные сортообразцы фасоли, обладающие высокой способностью к накоплению каллусной биомассы и формированию морфогенного каллуса: «Актатти», «Ред Гойя», «Журавушка» и «Камелия». Разработанный протокол получения морфогенных каллусных культур фасоли может быть использован в качестве альтернативного биотехнологического подхода для выделения лектинов и получения на их основе биопрепаратов для сельского хозяйства.

Ключевые слова: фасоль, каллусные культуры, оптимизация питательных сред, 2,4-Д, НУК, морфогенный каллус, лектины, биопрепараты.

Во всем мире проблема продовольствия остается одной из главных и насущных на сегодняшний день. В связи с этим в развитых сельскохозяйственных странах большое внимание уделяется разработке биологических и экологически безопасных методов защиты растений, направленных на сокращение объемов использования ядохимикатов [1, 2]. По химическому составу семена фасоли уникальны и включены в группу важных продуктов, обеспечивающих население полноценным белком. Однако белковый комплекс фасоли содержит ряд токсичных и антиалиментарных факторов питания, блокирующих активность пищеварительных ферментов, которые, возможно, принимают участие в защитных механизмах растений. Наличие антипитательных веществ (ингибиторов гидролаз, лектинов и цианогенных гликозидов) с высокой активностью в семенах фасоли делает ее перспективной с точки зрения биотехнологической переработки и получения фитопрепаратов для защиты растений, направленных на сокращение объемов использования ядохимикатов [3–5].

По оценкам ученых, с 2014 по 2019 гг. рост рынка биоудобрений возрос более чем в 1,6 млрд долл. Связано это с увеличением числа предприятий органической пищевой промышленности, их потребления и осведомленностью общества в отношении здоровья и угроз, возникающих в результате применения химических веществ в сельском хозяйстве [6]. Однако в настоящее время использование и применение биологического метода защиты растений в Казахстане развиты недостаточно. Сегодня создан и успешно применяется целый спектр микробиологических препаратов для нужд промышленности, сельского хозяйства, животноводства и охраны окружающей среды. В то же время разработка и производство биопрепаратов растительного происхождения находятся на начальном этапе. В Казахстане производится лишь 2 % биопрепаратов от мирового производства, несмотря на то, что наша республика обладает большим рыночным потенциалом для их эффективного применения в таких областях, как нефтегазовый сектор, сельское хозяйство, медицина, животноводство и др. Потребность в биопрепаратах для Казахстана имеет большое народно-хозяйственное значение, поскольку около 9000 га

земель сельскохозяйственных угодий заражено вредителями, более 2,5 млн га — сорными растениями; распространено около 70 видов болезней микробного происхождения [7].

Современным направлением для создания экологически безопасных комплексных биопрепаратов является разработка методов культивирования изолированных клеток и тканей на искусственных питательных средах. Во многом продуктивность культивируемых клеток значительно превышает производительность растений для выделения биологически активных веществ, ценных для медицины, сельского хозяйства, пищевой промышленности. Кроме того, получение веществ вторичного биотехнологического синтеза из каллусных культур создает возможность использования растений, не произрастающих в данных природных условиях, и способствует проведению исследований в течение всего года [8].

В связи с этим актуальным представляется получение каллусных культур бобовых с максимальным содержанием комплекса биологически активных веществ. К ним относятся, например, лектины фасоли, которые принимают активное участие в защитных реакциях растений против насекомых, вредителей и поражения фитопатогенными грибами [9–12]. Ранее в наших работах проведен анализ содержания лектинов в коллекции фасоли, представленной сортообразцами местной и зарубежной селекции [13]. Биоскрининг позволил определить перспективные сортообразцы, характеризующиеся повышенным содержанием лектинов. Многие бобовые уже введены в культуру тканей и имеют высокую генотипическую специфичность [14]. Получение каллусных культур — важный инструмент в биотехнологии растений, который позволяет использовать клеточную биомассу для производства различных метаболитов и биологически активных веществ [15].

Таким образом, цель данного исследования — разработка протокола массового получения каллусных культур фасоли, являющихся альтернативным источником получения лектинов, используемых в производстве биопрепаратов для сельского хозяйства.

Методы и материалы

В качестве объектов исследования использовали 6 сортообразцов фасоли зерновых бобовых культур (семейство *Fabaceae*) казахстанской, российской и зарубежной селекции: «Актатти» (Казахстан), «Бийчанка» (Россия), «Журавушка» (Россия), «Иранская» (Иран), «Камелия» (США) и «Ред Гойя» (США). Семена исследуемых образцов были получены из коллекции агробиостанции КазНУ им. аль-Фараби. В качестве эксплантов использовали эпикотили и гипокотили 7–14-дневных асептических проростков. Для получения асептических проростков семена промывали теплой проточной водой и выдерживали в растворе хозяйственного мыла в течение 30 мин. Затем семена отмывали от мыльного раствора и стерилизовали 10 мин в 20 %-ном гипохлориде натрия с 2–3 каплями TWIN 20; 5 мин — в 5 %-ном H_2O_2; 5 мин — в 70 %-ном спирте и трижды промывали стерильной дистиллированной водой. Семена проращивали в пробирках на безгормональной питательной среде Мурасиге-Скуга в инкубаторе при 24–26 °С.

Для получения первичной культуры каллусов экспланты размером 2–3 см культивировали на средах Ушимия-Мурасиге [16], Мурасиге-Скуга [17] и Гамборга-Эвеленга В 5 [18]. Экспланты высаживали на чашки Петри по 10 эксплантов на каждую чашку. Питательные среды автоклавировали при 121 °С и 1 атм. в течение 20 мин. В качестве индукторов каллусогенеза использовали ауксины (2,4-Д и НУК) и цитокинины (кинетин). Каллусы культивировали на свету при температуре 23–25 °С, 16-часовом фотопериоде с интенсивностью освещения 10000 люкс. Эксперименты были выполнены в трехкратной повторности. Начало каллусообразования наблюдали через 7–10 дней. Процесс каллусогенеза оценивали по трем показателям: 1) частота каллусогенеза — доля эксплантатов, образовавших каллус; 2) интенсивность каллусообразования — степень накопления каллусной массы; 3) морфологический тип каллуса. Интенсивность каллусообразования оценивалась по балльной системе: 0 — отсутствие каллуса; 1 — каллусогенез охватывает до половины экспланта; 2 — каллусной тканью покрыто до 2/3 экспланта; 3 — эксплант виден лишь частично; 4 — эксплант полностью покрыт каллусом.

Индекс роста каллусных культур определялся через каждые 28 дней культивирования и вычислялся по формуле

$$I = \frac{Wo - Wt}{Wo},$$

где *Wo* — начальная масса каллуса, г; *Wt* — масса каллуса в конце цикла выращивания, г.

Для наращивания каллусной биомассы каллусы пассировали каждые 28 дней на питательной среде того же минерального и гормонального состава. Для пассирования брали кусочки каллусной

ткани весом не более 7–10 г. Для получения достаточного количества каллусной биомассы проводили 5–7 пассажей для каждого сортообразца. Изучение влияния гормонального состава среды на прирост клеточной биомассы и получение морфогенных каллусов проводили на средах с 2,4-Д и НУК в концентрациях 2, 4, 6 и 8 мг/л. Концентрация кинетина составляла 0,25 мг/л. Статистический анализ был выполнен с использованием программы однофакторного дисперсионного анализа ANOVA и программы STATISTICA.

Результаты и их обсуждение

Индукция каллусогенеза. На первом этапе исследований проводили изучение влияния минерального состава питательной среды на индукцию каллусогенеза. Для получения каллусной биомассы эксплanты (эпикотиль и гипокотиль) культивировали на питательных средах различного минерального состава, содержащие 2 мг/л 2,4-Д и 0,25 мг/л кинетина. В исследование были включены наиболее часто используемые для культивирования бобовых культур питательные среды: Ушимия-Мурасиге (УМ), Мурасиге-Скуга (МС) и Гамборга-Эвеленга (В5). Установлено, что частота и интенсивность каллусогенеза зависят от состава питательной среды и типа экспланта. Сравнительный анализ зависимости частоты каллусогенеза от типа экспланта показал, что оба экспланта обладают каллусообразующей способностью, причем на всех питательных средах наибольшая каллусообразующая способность отмечена на экспланте эпикотиль, хотя индукция каллусогенеза на экспланте гипокотиль начиналась на 2–3 дня раньше (табл. 1).

Таблица 1

Частота каллусогенеза у сортообразцов фасоли на различных питательных средах, %

Сортообразцы фасоли	Питательные среды, экспланты					
	УМ		МС		В5	
	эпикотиль	гипокотиль	эпикотиль	гипокотиль	эпикотиль	гипокотиль
«Актатти»	100 ±1,40	90±1,24	92±1,34	80±1,25	35±0,80	30±0,64
«Бийчанка»	95±1,28	84±1,22	90±1,28	68±1,16	30±0,56	25±0,40
«Иранская»	85±1,34	80±1,17	88±1,35	76±1,18	28±0,40	20±0,30
«Камелия»	97±1,20	82±1,14	90±1,15	82±1,25	32±0,34	30±0,33
«Ред Гойя»	100±1,17	87±1,16	95±1,14	84±1,30	35±0,44	28±0,39
«Журавушка»	90±1,23	85±1,14	87±1,19	75±1,12	32±0,56	26±0,42

В результате проведенных исследований установлена зависимость частоты каллусогенеза от минерального состава питательной среды. Наибольшая частота каллусообразования наблюдалась на среде УМ и составляла на экспланте: эпикотиль 85–100 %, гипокотиль — 82–90 %.

На питательной среде МС частота образования каллусов была тоже высокой, но ниже, чем на среде УМ. На среде В5 частота каллусогенеза была самой низкой и не превышала 35 %. Наибольшая интенсивность каллусогенеза также отмечена при культивировании на среде УМ. На средах МС и В5 интенсивность каллусогенеза не превышала 3,45 и 1,84 балла соответственно (табл. 2).

Таблица 2

Интенсивность каллусогенеза у сортообразцов фасоли на различных питательных средах, баллы

Сортообразцы фасоли	Питательные среды, экспланты					
	УМ		МС		В5	
	эпикотиль	гипокотиль	эпикотиль	гипокотиль	эпикотиль	гипокотиль
«Актатти»	4,0 ±0,48	3,77±0,45	3,20±0,89	2,80±0,72	1,84±0,70	1,45±0,26
«Бийчанка»	3,27±0,98	3,12±0,58	3,25±0,45	2,70±0,30	1,35±0,75	1,28±0,95
«Иранская»	2,45±0,38	2,30±0,78	2,72±0,65	2,70±0,30	1,45±0,46	1,35±0,62
«Камелия»	3,75±0,48	3,42±0,78	2,58±0,88	2,15±0,28	1,44±0,46	1,15±0,18
«Ред Гойя»	4,0 ±0,52	3,50±0,44	3,45±0,28	3,25±0,45	1,72±0,69	1,45±0,68
«Журавушка»	3,12±0,52	2,35±0,35	2,52±0,98	2,41±10,42	1,13±0,43	1,12±0,40

Наибольшая каллусообразующая способность изученных сортообразцов фасоли на среде УМ, по-видимому, связана с тем, что содержание витаминов B_1, B_6 и РР в среде УМ, по сравнению со средой

МС, увеличено в 10 раз. Среды УМ и МС имеют одинаковый минеральный состав [16, 17], в связи с чем каллусообразующая способность изученных сортообразцов на данных питательных средах отличается незначительно. Поскольку среда В5 имеет другой состав макро- и микроэлементов по сравнению с УМ, МС, можно предположить, что каллусообразующая способность зависит не только от содержания витаминов, но и от минерального состава.

Индекс роста каллусных культур связан также с минеральным составом питательных сред. На питательной среде УМ индекс роста варьировался от 3,2 до 6,9. Максимальная скорость роста отмечена для сортообразцов «Ред Гойя» и «Актатти», минимальная — для сорта «Иранская». На средах МС и В5 индекс роста не превышал 2,0 и 1,7 соответственно (рис. 1).

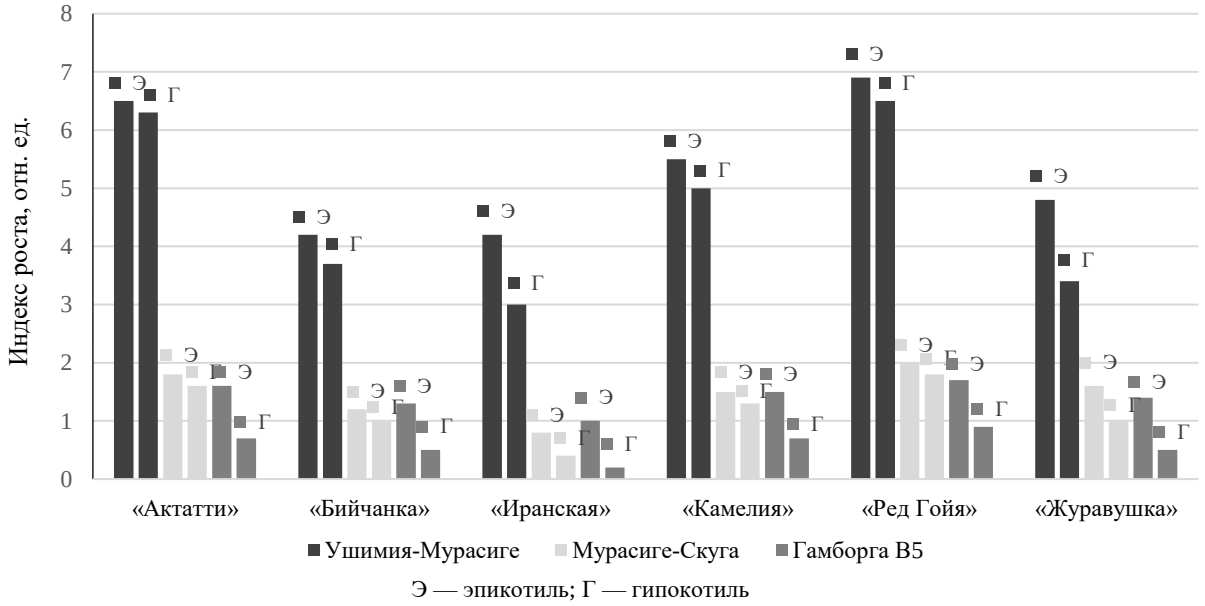


Рисунок 1. Зависимость индекса роста каллусной культуры от минерального состава питательной среды

Среди изученных образцов можно выделить «Актатти» и «Ред Гойя», для которых частота и интенсивность каллусогенеза были максимальными на обоих эксплантах.

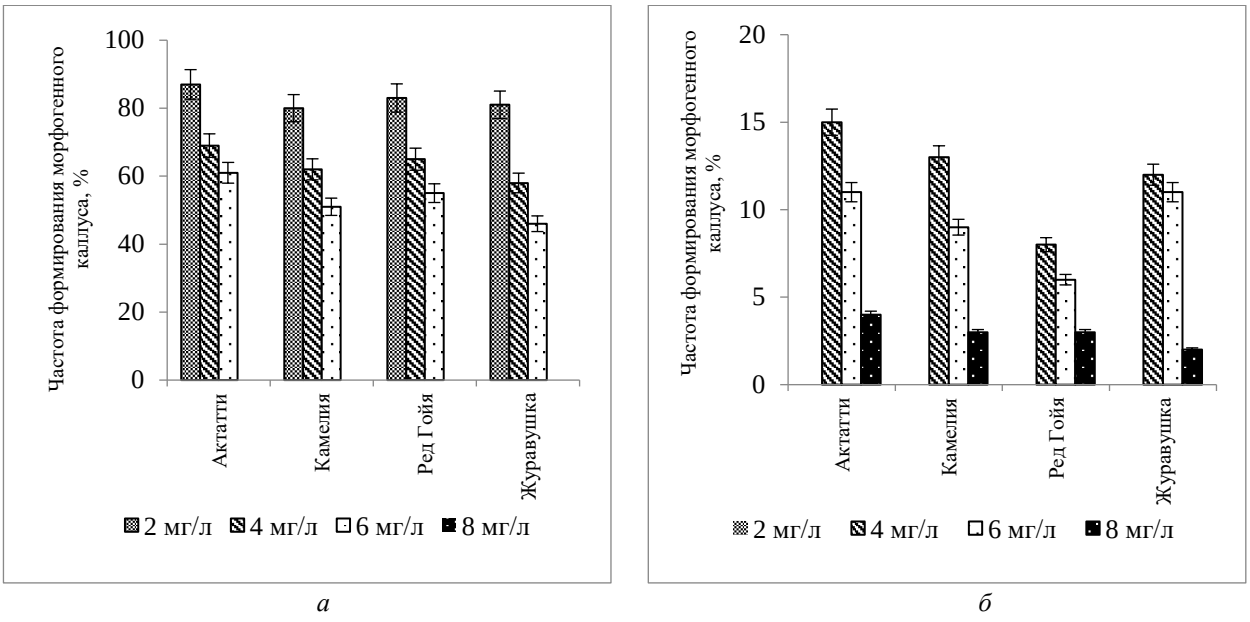
Морфологическая структура каллуса определялась визуально по общепринятой методике [19]. При культивировании на среде УМ и МС формировался бело-желтый, глобулярный, рыхлый каллус с хлорофилл-содержащими участками, которые представляют собой зоны морфогенеза. Данный тип каллуса был определен как морфогенный. На среде В5 формировался плотный, компактный, малообводненный каллус, который был охарактеризован как неморфогенный. Изучение влияния минерального состава на индукцию каллусогенеза показало, что оптимальной средой является среда Ушимия-Мурасиге. Основа данной среды — среда Мурасиге-Скуга, которая чаще всего использовалась для индукции каллусных культур различных видов фасоли и других бобовых культур [20, 21].

Оптимизация гормонального состава питательных сред для получения клеточной биомассы различных сортообразцов фасоли

В данной серии экспериментов изучено влияние двух видов ауксинов (2,4-Д и НУК) в концентрациях 2, 4, 6 и 8 мг/л на прирост клеточной биомассы и получение морфогенных каллусов. В результате изучения 6 сортообразцов фасоли показано, что частота формирования морфогенного каллуса зависит от типа и концентрации фитогормонов. При концентрации 2,4-Д 2 мг/л выход морфогенного каллуса был наибольшим и варьировался от 80 до 87 %. Увеличение концентрации 2,4-Д до 4 мг/л и 6 мг/л незначительно влияло на прирост биомассы, но сопровождалось снижением частоты образования морфогенных каллусов. Концентрация 2,4-Д 8 мг/л оказалась летальной для каллусных культур всех образцов фасоли.

При использовании в качестве индуктора НУК частота каллусогенеза была гораздо ниже, чем на средах с 2,4-Д. Максимальный прирост каллусных культур отмечен при концентрации НУК 4 мг/л, однако, в зависимости от генотипа, частота каллусогенеза не превышала 20–40 %. При этом доля морфогенных каллусов составляла не более 15 %. При концентрации НУК 2 мг/л индукции каллусогенеза

не наблюдалось. Увеличение концентрации НУК в среде культивирования до 6 мг/л и 8 мг/л вызывало резкое снижение частоты каллусогенеза и формирование ризогенного каллуса. В результате исследований нами также выявлены сортовые различия по способности к формированию морфогенного каллуса. Морфогенные структуры формировались на каллусных культурах четырех сортообразцов: «Актатти», «Ред Гойя», «Журавушка» и «Камелия». Максимальная частота образования морфогенных каллусов отмечена для сорттообразцов «Актатти» (87 %) и «Ред Гойя» (83 %) (рис. 2).



а *б*

Рисунок 2. Частота формирования морфогенных каллусов в зависимости от концентрации 2,4-D (*а*) и НУК (*б*)

В большинстве исследований по изучению процессов индукции морфогенных каллусных культур фасоли показано, что в качестве индуктора эмбрио- и органогенеза чаще всего используют БАП и TDZ [22–24]. В работе A.M. Gatica с соавторами показано, что у костариканских сортов фасоли формирование эмбриогенных каллусов зависело от концентрации БАП и аденин сульфата, но генотипической зависимости установлено не было. Независимо от сорта, среда, содержащая 5 мг/л БАП и 20–40 мг/л аденин сульфата, способствовала индукции образования побегов [25]. В наших исследованиях установлено, что у изучаемых сортообразцов фасоли частота формирования морфогенных каллусов зависела от типа фитогормонов и концентрации 2,4-Д. Изучение влияния различных видов и концентраций ауксинов показало, что наиболее интенсивно морфогенные каллусы формировались на экспланте эпикотиль на среде, содержащей 2 мг/л 2,4-Д.

Концентрация 2,4-Д 8 мг/л оказалась летальной для каллусных культур фасоли. Предполагается, что при высоких концентрациях 2,4-Д сильно увеличивается скорость образования этилена и уменьшается скорость растяжения клеток. Вероятно, у двудольных растений подавление роста высокими концентрациями ауксина опосредовано их действием на синтез этилена [26]. Использование НУК не дало положительных результатов.

Ряд исследователей отмечают связь морфологии каллусов с их способностью к регенерации растений и синтезу биологически активных веществ. Культивирование каллусной ткани в условиях *in vitro* вызывает изменение метаболической активности клеток и выражается в модификации количества и состава биологически активных веществ в каллусной ткани по сравнению с исходным растением [27, 28]. В работе И.Ф. Шаяхметова показано, что морфогенный каллус отличается высоким содержанием лектинов, что является следствием морфогенетических процессов [29].

Ранее проведенные нами исследования по оценке лектиновой активности перспективных сортоформ фасоли показали, что сорта «Журавушка» и «Актатти» отличаются наиболее высоким содержанием лектинов [13]. Результаты, полученные в ходе настоящих исследований, позволили определить, что сортообразцы «Актатти», «Журавушка» и «Ред-Гойя» являются наиболее перспективными для получения каллусных культур и могут в дальнейшем применяться для разработки биотехнологических подходов выделения лектинов.

Таким образом, разработанный протокол массового получения каллусных культур может быть предложен в качестве альтернативного пути для выделения лектинов фасоли и получения на их основе биопрепаратов для повышения устойчивости растений к различным биотическим и абиотическим факторам.

Заключение

Современным направлением для создания экологически безопасных комплексных биопрепаратов является их получение на основе культуры клеток и тканей растений. Данная работа посвящена разработке протоколов массового получения каллусных культур фасоли казахстанской и зарубежной селекции. На основе оптимизации минерального и гормонального состава питательных сред разработан протокол получения морфогенных каллусных культур перспективных сортообразцов фасоли, который может быть использован в качестве альтернативного биотехнологического подхода для выделения лектинов и получения на их основе биопрепаратов для сельского хозяйства.

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Қазақстандық және шетелдік селекция үрмебұршақтарынан каллус дақылын жаппай алудың тәсілін өңдеу

Экологиялық қауіпсіз кешенді биопрепараттарды жасаудың қазіргі заманғы бағыттарының бірі — оларды өсімдіктердің жасушалары мен ұлпалары негізінде алу болып табылады. Көп жағдайда медицина, ауыл шаруашылығы, тамақ өнеркәсібі үшін өсірілетін жасушалардың өнімділігі құнды биологиялық белсенді заттарды бөліп алуға қолданылатын өсімдіктердің өнімділігінен едәуір асып түседі. Зерттеудің мақсаты — ауылшаруашылығы үшін биопрепараттар өндіруде қолданылатын лектиннің баламалы көзі ретінде үрмебұршақтың каллустық дақылын жаппай алу хаттамасын өңдеу. *In vitro* дақылына қазақстандық және шетелдік селекциядағы үрмебұршақтың алты сорт үлгілерін енгізу шарттары оңтайландырылған, каллусогенез индукциясы үшін қоректік орталардың оңтайлы құрамы белгіленген, жоғары каллус түзушілік қабілеті бар сорт үлгілері табылған. Каллусогенез жиілігінің қоректік ортаның минералдық құрамына және эксплант түріне байланыстылығы анықталды. Каллус түзудің ең үлкен жиілігі Ушимия-Мурасиге ортасында (80–100 %) байқалды, ал каллус индукциясы үшін ең қолайлы эксплант эпикотиль болып табылады. Морфогенді каллустардың қалыптасу жиілігі фитогормондардың түрі мен концентрациясына байланыстылығы көрсетілген. Морфогенді каллустардың пайда болуы тек 2,4-Д қатысуымен байқалған. Морфогенді каллустың ең жоғары нәтижесі 2,4-Д 2 мг/л концентрациясында табылған. Каллус биомассасының жиналуына және морфогенді каллус қалыптастыруға жоғары қабілетті үрмебұршақтың перспективалы сұрыптық үлгілері: «Ақтәтті», «Ред Гойя», «Журавушка» және «Камелия» анықталды. Дайындалған үрмебұршақтың морфогенді каллус дақылдарын алу хаттамасы лектиндерді бөлу және олардың негізінде ауыл шаруашылығы үшін биопрепараттар алудың баламалы биотехнологиялық тәсілі ретінде пайдаланылуы мүмкін.

Кілт сөздер: үрмебұршақ, каллус дақылы, қоректік орта құрамын оңтайландыру, 2,4-Д, НСҚ, морфогендік каллус, лектиндер, биопрепараттар.

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Development the protocol of mass production calluscultures of beans of Kazakhstan and foreign selection

A modern direction for the creation of environmentally friendly integrated biological products is their preparation based on the plant cell and tissue cultures. To a large extent, the productivity of cultured cells significantly exceeds the productivity of plants for the isolation of biologically active substances valuable for medicine, agriculture, and the food industry. The purpose of this study is to develop a protocol for the mass production of

callus beans, which are an alternative source of lectins used in the production of biological products for agriculture. The conditions for introducing six varieties of beans of Kazakhstan and foreign breeding into the *in vitro* culture were optimized, the optimal composition of nutrient media for the induction of callusogenesis was established, varieties with high callus-forming ability were determined. The dependence of the frequency of callusogenesis on the mineral composition of the nutrient medium and the type of explant has been established. The highest frequency of callus formation was observed on the UM medium (80–100 %), and the most suitable explant for the induction of callus is epicotyl. The dependence of the frequency of morphogenic callus formation is shown, depending on the type and concentration of phytohormones. The formation of morphogenic callus was observed only in the presence of 2,4-D. The maximum yield of morphogenic callus was noted at a 2 mg/l 2,4-D. Promising varieties of beans with a high ability to produce callus biomass and the formation of morphogenic callus have been identified: "Actatt", "Red Goya", "Zhuravushka" and "Camellia". The developed protocol for the production of morphogenic callus bean cultures can be used as an alternative biotechnological approach for the isolation of lectins and the production of biological products based on them for agriculture.

Keywords: beans, callus cultures, optimization of the nutrient media, 2,4-D, NAA, morphogenic callus, lectins, biological products.

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Analysis of representatives of *Lamiaceae* family in the flora of the Central Kazakhstan

The work presents the results of research of *Lamiaceae* family's plants of flora of vascular plants of the Central Kazakhstan (Karaganda region). Studies have found the growth of 48 species belonging to 20 genera. This composition is 29.6 % of the total number of species and 42.6 % of the total number of genera of Kazakhstan's flora. The largest number of species recorded in the genera *Thymus* and *Scutellaris*. In the territory of the Central Kazakhstan there is a growth of 6 endemics (*Hyssopus ambiguus, Lagochillus acutilobus, Thymus lavrenkoanus, Th. crebrifolius, Th. rasitatus, Th.eremita*), 9 species are defined as objects for protection in nature. Life forms are dominated by perennial herbaceous plants, and environmental groups include mesophytes and xerophytes. Among members of *Lamiaceae* family are allocated 9 practical-useful groups: fodder — 28 species, technical — 6, medicinal — 32, honey — 46, ornamental-decorative — 17, food — 7, vitamin — 5, essential-oil — 43, insecticidal — 6. The distribution of species in the territory of Karaganda region, which depends on soil and climatic conditions, has been determined.

Keywords: *Lamiaceae* family, flora, Karaganda region, distribution, life forms, ecological groups, endemics, гические группы, эндемики, phytosecurity status, practical-used species.

Introduction

The study of flora of certain regions of the Republic of Kazakhstan has important theoretical and applied significance. The last comprehensive studies on flora were carried out in the 60–70 years of the 20th century [1], so it is necessary to study the flora of separated regions with a detailed characteristic of taxonomic groups.

Our attention has been drawn to the family *Lamiaceae*, many species of which have value as virtually potential valuable species [2–5]. For this family in the flora of Central Kazakhstan there is no full data on the full species and morphological composition, there is no information on the general distribution of species, places of localization and phytoprotective status [6]. It is of great importance to identify promising species, as this will allow to fully exploring the possibilities of using individual species in different industries.

The study of plant resources of certain regions of the Republic will allow creating a complete characteristic of valuable and rare plants, which will ensure the preservation of species diversity [7]. New botanical data and comprehensive research on selected species will allow them to be applied in new fields of science and industry, providing the local population with the necessary plant stocks.

The aim of present research is to carry out the analysis of family *Lamiaceae* of flora of the Karaganda region, including the taxonomical analysis, the analysis of vital forms and ecological groups, distribution, the phytosecurity status and economic and valuable properties.

Objects and methodology

The material for carrying out the research was the herbal duties stored in the herbarium funds of JSC "Scientific and production holding "Phytochemistry", Department of Botany of Ye.A. Karaganda State University, Zhezkazgan Botanical Garden and Zhezkazgan University named after O. Baikonurov, as well as the results of its own long field.

The species of the family *Lamiaceae* have become objects of study. Field studies in the territory of Karaganda region have been carried out by route method with the aim of the most complete detection of taxonomic composition, study of their areas, peculiarities of ecological development and biological factors [8]. Determination of species was carried out according to conventional determinants [1, 9–11], names of taxa were indicated in accordance with S.K. Cherepanov's summary [12].

The species belonging to the ecological group was determined in relation to the humidification conditions [13], life forms were indicated by the method of I.G. Serebryakov [14].

The allocation and justification of the status of rare endangered species of the region was carried out on the basis of the own materials and works of M.S. Baytenov [15], the Red Book of Kazakhstan (2014) [16], the list of rare and endangered plants of Karaganda region [17]. The status of the species characterizes the state of populations in nature and corresponds to the designations adopted in the IUCN Plant Red Data Book (1978):

1 (E) are species found in single instances, known from one or two or more places, endangered — endangered species.

2 (V) are species whose populations are declining due to natural causes or human-induced effects of habitat change (destruction) and other factors — vulnerable species. These species are not directly threatened with extinction, but are found in either small numbers or limited territories and in specific ecological niches.

3 (R) are species whose distribution is restricted to small territories or scattered in significant territories not currently endangered, but whose numbers are declining — shrinking species (10 species).

Practical-useful groups of species are identified according to the data of scientific publication [18–26].

Results and discussion

The territory of the Central Kazakhstan (Karaganda region) is located within the continental West Siberian steppe zone and occupies a middle position in the republic. The following floristic districts are located on the territory of the Central Kazakhstan: Western small-scale miner, Eastern small-scale miner, Ulytau, Kararaly, Betpakdala [27, 28]. The climate of the Central Kazakhstan is sharply continental. Summers are hot and dry; winters are low-white, harsh, with winds and burans. The territory of the region is almost all year round in the region of high pressure.

Taxonomic analysis. At the present stage, the family *Lamiceaea* of the Central Kazakhstan's flora is represented by 48 species belonging to 20 genera (Table 1), which is 29.6 % of the total number of species of the flora family of Kazakhstan and 42.6 % of the total number of genera.

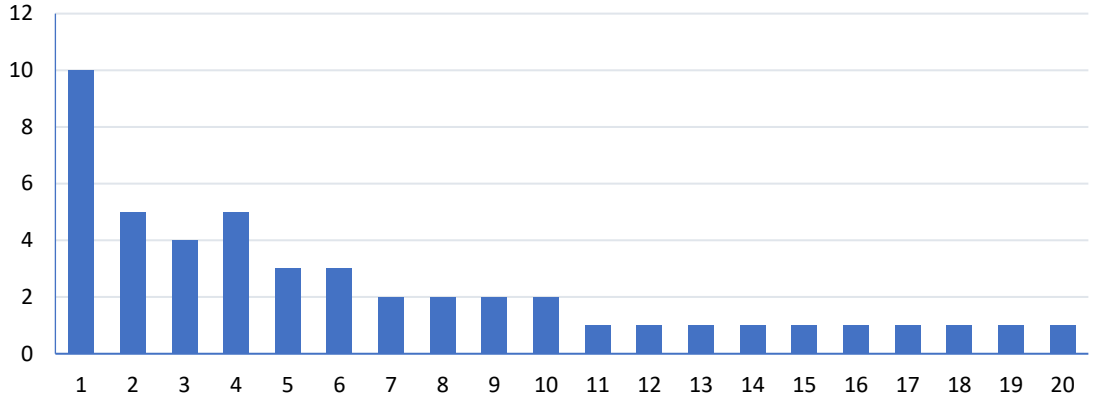
Table 1

Taxonomical structure *Lamiceaea* family in the flora of the Central Kazakhstan

№	Genus	Total number of species in the Central Kazakhstan, pieces	Total number of species in Kazakhstan, pieces	% from total number of species in Kazakhstan
1	*Thymus* L.	10	22	45.5
2	*Scutellaria* L.	5	35	14.3
3	*Dragocephalum* L.	4	20	20.0
4	*Nepeta* L.	5	14	35.7
5	*Mentha* L.	3	8	37.5
6	*Phlomoides* Moench.	3	4	75.0
7	*Galeopsis* L.	2	3	66.7
8	*Hyssopus* L.	2	4	50.0
9	*Lycopus* L.	2	2	100.0
10	*Salvia* L.	2	8	25.0
11	*Ziziphora* L.	1	7	14.3
12	*Glechoma* L.	1	1	100.0
13	*Lagochillus* Bunge	1	16	6.3
14	*Lallemantia* Fisch. et Mey	1	1	100.0
15	*Lamium* L.	1	3	33.3
16	*Leonurus* L.	1	4	25.0
17	*Lophanthus* Adans.	1	3	33.3
18	*Prunella* L.	1	1	100.0
19	*Sideritis* L.	1	1	100.0
20	*Stachys* L.	1	5	20.0

By species diversity, 2 genera are central positions: *Thymus* L. and *Scutellaria* L.

So, genera *Thymus* L. in the flora of the Central Kazakhstan has 10 species: *Th.mongolicus* (Ronn.) Ronn., *Th.crebrifolius* Klok., *Th.eremita* Klok., *Th. guberlinensis* Iljin, *Th.kirgisorum* Klok., *Th. lavrenkoanus* Klok., *Th. marschallianus* Willd., *Th. minussinensis* Serg., *Th. rasitatus* Klok., *Th. roseus* Schipz. (Fig. 1).



By vertically — number of species (in pieces); genus: 1 — *Thymus*, 2 — *Scutellaria*, 3 — *Dragocephalum*, 4 — *Nepeta*, 5 — *Mentha*, 6 — *Phlomoides*, 7 — *Galeopsis*, 8 — *Hyssopus*, 9 — *Lycopus*, 10 — *Salvia*, 11 — *Ziziphora*, 12 — *Glechoma*, 13 — *Lagochillus*, 14 — *Lallemantia*, 15 — *Lamium*, 16 — *Leonurus*, 17 — *Lophanthis*, 18 — *Prunella*, 19 — *Sideritis*, 20 — *Stachys*

Figure 1. Distribution of species in genera of *Lamiceae* family of the Central Kazakhstan's flora

Genus *Scutellaria* L. consists from 5 species: *S. dubia* Taliev et Sirj., *S. galericulata* L., *S. grandiflora* Sims., *S. scordiifolia* Fisch. ex Schrank., *S. supina* L.

The most of the genera are arranged in descending order as follows:

– genus *Dragocephalum* L. — 4 species (*D. nutans* L., *D. peregrinum* L., *D. ruyschiana* L., *D. thymiflorum* L.);
– genus *Nepeta* L. — 4 species (*N. cataria* L., *N. micrantha* Bunge, *N. pannonica* L., *N. ucranica* L.);
– genus *Mentha* L. — 3 species (*M. arvensis* L., *M. longifolia* (L.) Huds., *M. micrantha* (Fisch. ex Benth.) Litv.);
– genus *Phlomoides* Moench. — 3 species (*Ph. agraria* (Bunge) Adyl., *Ph. puberula* (Kryl. et Serg.) Adyl., R. Kam. et Machmedov; *Ph. tuberosa* (L.) Moench.);
– genus *Galeopsis* L. — 2 species (*G. bifida* Boenn., *G. ladanum* L.);
– genus *Hyssopus* L. — 2 species (*H. ambiguus* (Trautv.) Iljin, *H. macranthus* Boriss.);
– genus *Lycopus* L. — 2 species (*L. europaeus* L., *L. exaltatus* L.);
– genus *Salvia* L. — 2 species (*S. nemerosa* L., *S. stepposa* Schost.);

The remaining genera are represented by only 1 species:

– genus *Ziziphora* L. (*Z. clinopodioides* Lam.).
– genus *Glechoma* L. (*G. hederacea* L.);
– genus *Lagochillus* Bunge (*L. acutilobus* (Ledeb.) Fisch. et Mey);
– genus *Lallemantia* Fisch. et Mey (*L. royleana* (Benth.) Benth.);
– genus *Lamium* L. (*L. amplexicaule* L.);
– genus *Leonurus* L. (*L. glaucescens* Bunge);
– genus *Lophanthus* Adans. (*L. schrenkii* Levin);
– genus *Prunella* L. (*P. vulgaris* L.);
– genus *Sideritis* L. (*S. montana* L.);
– genus *Stachys* L. (*S. palustris* L.).

Among the 48 species, there is a growth of 6 endemic species (*Hyssopus ambiguus, Lagochillus acutilobus, Thymus lavrenkoanus, Th. crebrifolius, Th. rasitatus, Th. eremita*), which is 14 % of the total species composition.

The largest species diversity of *Lamiaceae* family grows in the northern, north-eastern and central regions of Karaganda region (the Central Kazakhstan), smaller number of species grows in the southern and south-western regions, which is due to the existing difference in soil, temperature conditions and annual precipitation.

Regionally rare 8 species are proposed by us to be added to the list for protection (Table 2).

Table 2

List of the Central Kazakhstan rare vascular plants and the plants in need of protection

№	Species	Status	Condition of the population	Protection measures	Comment
1	*Thymus lavrenkoanus*	2(V)	Vulnerable specie	Monitoring the condition of the population. Protection of habitats	Endemic specie
2	*Th. crebrifolius*	3(R)	Rare specie	Protection of habitats	Endemic specie
3	*Th. eremita*	3(R)	Rare specie	Protection of habitats	Endemic specie
4	*Th. minussinensis*	2(V)	Vulnerable specie	Monitoring the condition of the population. Protection of habitats	Endemic specie
5	*Hyssopus macranthus*	3(R)	Rare specie	Monitoring the condition of the population. Protection of habitats	Endemic specie
6	*Glechoma hederacea*	1(E)	Endangered specie	Monitoring the condition of the population. Protection of habitats	Relict specie
7	*Lophanthus schrenkii*	3(R)	Rare specie	Protection of habitats	
8	*Prunella vulgaris*	1(E)	Endangered specie	Monitoring the condition of the population. Protection of habitats	Relict specie

Analysis of life forms. As a result of the ranking of plants by life forms, it was found that the following groups grow on the territory of the Central Kazakhstan: herbaceous perennials, annual, biennial and semi-shrub plants (Table 3).

Table 3

Distribution of *Lamiaceae* plants of the Central Kazakhstan's flora by life forms (according to I.G. Serebryakov)

Life form	Number of species, pieces	% from total number of species
Perennials herbaceous plants	27	56.3
Annual and biennial herbaceous plants	8	16.6
Semi-shrub plants	13	27.1
Total:	48	100.0

Most species in the flora of the Central Kazakhstan are represented by herbaceous plants — 35 species or 72.9 %. Share of perennials herbaceous plants is 27 species or 72,9 % (*Mentha arvensis, M.longifolia, Nepeta cataria, Nepeta pannonica, Nepeta ucranica, Lycopus europaeus, Phlomiodes tuberosa* and other); annual and biennial herbaceous species are 8 or 16,6 % (*Galeopsis bifida, Lamium amplexicaule, Galeopsis ladanum, Sideritis montana*).

The rest of the species belongs to semi-shrub life form — 13 species or 27,1 % (*Hyssopus ambiguus, H.macranthus, Thymus lavrenkoanus, Th.rasitatus, Th. minussinensis* and others).

Ecological analysis. Analysis of species by degree of alignment with different humidification conditions allowed to distinguish 5 groups: xerophytes and mesophytes — by 14 species, xeromesophytes — 5 species, mesoxerophytes — 6 species and hygrophytes — 9 species (Table 4).

Table 4

Distribution of *Lamiaceae* plants of the Central Kazakhstan's flora by ecological groups

Ecological group	Number of species, pieces	% from total number of species
Xerophytes	14	29.1
Xeromesophytes	5	10.4
Mesoxerophytes	6	12.5
Mesophytes	14	29.1
Hygrophytes	9	18.9
Total:	48	100.0

Mesophytes include species such as *Nepeta micrantha, Dragocephalum nutans, Lophanthus schrenkii, Phlomoides agraria*. Among xerophytes are *Thymus rasitatus, Th. crebrifolius, Hyssopus macranthus*. Among

hygrophytes are *Mentha arvensis, Mentha micrantha, Prunella vulgaris, Lycopus europaeus* and other. Group of xeromesophytes includes 5 species, among them *Ziziphora clinopodioides, Phlomoides tuberosa, Thymus marschallianus*. The remaining species are classified as mesoxerophytes.

Analysis of practical-useful plants. Studying of plants of *Lamiaceae* family have revealed that most species exhibit not one but several useful properties. Thus, mint species are fodder, decorative, food, medicinal, essential oil plants, contain vitamins, can be used to stain wool and fabrics.

It has been revealed that among 48 taxa 43 species are classified as essential oil plants, 32 species are medicinal plants, 46 species are honey plants, 28 species are fodder plants, 6 species are technical plants, 7 species are food plants, 17 species are decorative plants and 5 species are vitamin plants (Table 5).

Table 5

Distribution of *Lamiaceae* plants of the Central Kazakhstan's flora by practical-useful groups

Practical-useful group	Number of species, pieces	% from total number of species	Number of genera, pieces	% from total number of genera
Fodder	28	56	14	70
Technical	6	12	4	20
Medical	32	64	16	80
Honey	46	94	18	90
Ornamental-decorative	17	36	11	55
Food	7	14	6	30
Vitamin	5	10	4	20
Essential oily	43	86	17	85
Insecticide	6	12	4	20

Of greatest practical importance is the representative of the genera *Mentha, Thymus, Dragocephalum, Phlomis*.

Based on the extent and value of species, we have attempted to assess the possibilities of practical use of plants of *Lamiaceae* family, growing in the flora of the Central Kazakhstan.

Thus, pharmaceutical, food, perfumery and cosmetic industries are promising directions in developing Kazakhstan at the moment. One of the most important components for them is the use of their own renewable and environmentally friendly raw materials. As a supplement, the use of natural agents, that is, not of chemical origin, as the basis for the preservation of human health.

According to the available literary information [16–20], practical interest for medicine and pharmacy as medicinal plants has *Ziziphora clinopodioides, Thymus marschallianus, Th.crebrifolius, Th. rasitatus, Nepeta cataria, Nepeta ucranica*. These species are quite widespread in the territory of the Central Kazakhstan, there are semi-industrial reserves of raw materials.

Essential and other volatile compounds from *Nepeta, Thymus* and *Mentha* can be used to aromatize lollipops, candy, creams, soft drinks and liquor products.

Last researches [2, 4, 21] showed the possibility of using essential oils from *Hyssopus ambiguus, Glechoma hederaceae, Thymus roseus* for the pharmaceutical industry as the sources of antimicrobial and antifungal ointments, mixtures for inhalation, in aromatherapy, as well as for making flavors and flavorings.

Conclusion

Thus, 48 species of plants from Lamiaceae family grow in the natural flora in the territory of the Central Kazakhstan, which belonging to 20 genera, which is 29.6 % of the total number of species of the flora family of Kazakhstan and 42.6 % of the total number of genera.

Among the 48 species, 6 endemic species (*Hyssopus macranthus, Lagochillus acutilobus, Thymus lavrenkoanus, Th. crebrifolius, Th. rasitatus, Th. eremita*), which is 12.5 % of the total species composition.

The leading genera are *Thymus* and *Scutellaria*. The ecological spectrum is dominated by mesophytes and xerophytes; among the life forms are herbaceous perennial, annual and biennial plants. By practical-useful species the largest group belongs to medicinal, fodder, essential oil and honey plants. The largest species diversity of *Lamiaceae* family' plants locate in the northern, north-eastern and central regions of Karaganda region (the Central Kazakhstan), less diversity — in the southern and south-western regions, which is due to soil and climatic conditions.

The research work, analysis of literary data and herbal materials allowed for the first time to make a complete conspectus of the flora of *Lamiaceae* family of the Central Kazakhstan and maps of areas of identified plant species.

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М.Ю. Ишмуратова, М.К. Бутумбаева, Д.А. Таханова, К.Б. Жолдыбаева, М.М. Силантьева

Орталық Қазақстан флорасындағы Ерінгүлділер тұқымдасының өкілдерін талдау

Мақалада Орталық Қазақстанның (Қарағанды облысы) тамырлы өсімдіктер флорасының *Lamiaceae* тұқымдас өсімдіктерін зерттеу нәтижелері келтірілген. Негізгі жүргізілген зерттеулердің сараптамасы бойынша Орталық Қазақстанның флорасынан алынған өсімдіктердің 48 түрінің 20-сы ерінгүлділер тұқымдасына жатады. Бұл құрам түрлердің жалпы санының 29,6 % және тұқымдастардың жалпы санының 42,6 % құрайды. Осы 50 түрдің ішіндегі 6-ы эндемик өсімдік болып табылады (*Hyssopus ambiguus, Lagochillus acutilobus, Thymus lavrenkoanus, Th. crebrifolius, Th. rasitatus, Th. eremita*), 9 түрі қорғау объектілері ретінде анықталған. Осы таралған түрлердің ішінде маңызды орын алатыны *Thymus* L. және *Scutellaria* L. Зерттеу қорытындысы бойынша бұл аумақта өсімдіктердің бірнеше экологиялық тобы айқындалды, олардың ішінде көп кездесетіні мезофиттер және ксерофитттер. Олар көбінесе көпжылдық мен бір жылдықтар шөптесін өсімдіктер. Бұл аумақта Ерінгүлділердің 9 экономикалық құнды топтары анықталған, олар: дәрілік — 32, жемшөп — 28, техникалық — 6, эфир майы — 43, бал өсімдіктер — 46, қолданбалы — 17, дәрумендi — 5, тамақ — 7, инсектицидтер — 6. Топырақ пен климаттық жағдайларға байланысты Қарағанды облысының аумағында түрлердің таралуы анықталған.

Кілт сөздер: *Lamiaceae* тұқымдасы, флора, Қарағанды облысы, таралуы, тіршілік формасы, экологиялық топтар, эндемиктер, экономикалық құнды түрлер.

М.Ю. Ишмуратова, М.К. Бутумбаева, Д.А. Таханова, К.Б. Жолдыбаева, М.М. Силантьева

Анализ представителей семейства *Губоцветные* во флоре Центрального Казахстана

В статье приведены результаты исследования растений семейства *Lamiaceae* флоры сосудистых растений Центрального Казахстана (Карагандинская область). Установлено произрастание 48 видов, относящихся к 20 родам. Данный состав составляет 29,6 % от общего числа видов и 42,6 % от общего числа родов. Наибольшее число видов зафиксировано в родах *Thymus* и *Scutellaris*. На территории Центрального Казахстана отмечено произрастание 6 эндемов (*Hyssopus ambiguus, Lagochillus acutilobus, Thymus lavrenkoanus, Th. crebrifolius, Th. rasitatus, Th. eremita*), 9 видов определены в качестве объектов для охраны. Среди жизненных форм преобладают многолетние травянистые растения, в экологических группах — мезофиты и ксерофиты. Среди представителей семейства *Губоцветные* выделено 9 хозяйственно-ценных групп: кормовые — 28 видов, технические — 6, лекарственные — 32, медоносные — 46, декоративные — 17, пищевые — 7, витаминные — 5, эфирно-масличные — 43, инсектицидные — 6. Определено распространение видов по территории Карагандинской области, которое зависит от почвенных и климатических условий.

Ключевые слова: семейство *Lamiaceae*, флора, Карагандинская область, распространение, жизненные формы, экологические группы, эндемики, фитоохранный статус, хозяйственно-ценные виды.

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Scientific and legal aspects of preservation of rare representatives of dwarf almond section of flora in East Kazakhstan

This work includes the review of materials and different sources for the presence of chronological information on representatives of *Chamaeamygdalus* (*Amygdalus ledebouriana* and *Amygdalus nana*) and its legal status. Information analysis methods in various literature sources, such as illustrations in scientific articles and etc., were used in this study. Legal aspects of preservation (laws, decrees and legal acts, that have direct or indirect connection with the research topic, object of research and its preservation at the state level) of dwarf almond representatives were studied for the first time in Kazakhstan. The uniqueness of this work is the integration of interdisciplinary researches in the field of botany and law (environmental law) into the study of an object. Samples collected on the territory of East Kazakhstan region and various literature sources were used as material for this study. The results are presented in the form of a comparative table and illustration with notes and links to competent sources. The results of the study can be used for species identification and development of preserving actions for the rare plant species *Amygdalus ledebouriana* Schlecht., as well as for the training of specialists in the field of environmental protection in East Kazakhstan and for the development of new legal standards for the conservation of rare and endangered plant species. The studied herbarium samples of *A. ledebouriana* (IPBB) were transferred to the herbarium fund in the Institute of Botany and Phytointroduction (Almaty).

Keywords: almond, *Chamaeamygdalus*, *Amygdalus ledebouriana*, *Amygdalus nana*, plant preservation, plant identification, taxonomy.

Introduction

The floral composition of East Kazakhstan is rich in species diversity with various genetic and ecotypic forms of higher and lower plants that need to be studied. Two very close almond species — *Amygdalus ledebouriana* Schlecht. and *Amygdalus nana* L. grow on the territory of East Kazakhstan region. Both species belong to the section of dwarf almonds (*Chamaeamygdalus* Spach.). *A. ledebouriana* is a rare and endangered species (Figure 1), while *A. nana* is widespread on the territory of the Eurasian continent (steppe zone) [1].

In the European plant systematics, *Amygdalus* L. is not a separate genus, it is considered as the subgenus *Prunus* subgen. *Amygdalus* L. in Kazakhstan, according to the USSR's systematic of flora (1941), *Amygdalus* L. is an individual genus of the subfamily *Prunoideae*, family *Rosaceae.*

The plant called *A. ledebouriana* was identified and described for the first time by German botanist and mycologist Diederich Franz Leonhard von Schlechtendal. The first materials on that species were published in 1854: Schlecht. in Abh. Naturf. Ges. Halle 2 [2]. The species was described in the 10th volume of USSR Flora [3], in the 4th volume of Flora of Kazakhstan [4] and in the first part of the illustrated identifier of plants in Kazakhstan (Fig. 2). *A. ledebouriana* is included in the Red Book of Kazakhstan as rare and endangered plant species [5]. In all abovementioned sources, *A. ledebouriana* is positioned as a separate plant species with the narrow endemic characteristics.

The second species (*A. nana*) was firstly described by Carl Linnaeus in 1753. The first mentions of this species were in Sp. pl. [6]. There is a full description of *A. nana* in the 10th volume of USSR Flora published in 1941 [7]. For the book Flora of Kazakhstan, this species was described 1961 in the 4th volume [8]. The major differences between two species are ecological area of growing and the shape of seed (seeds of *A. ledebouriana* are aslant-elongated).

Similarity of two species and different approaches in systematic of the genus create the problems for identification of plant samples. Ambiguity of systematic also inhibits processes of its preservation and rational

use. In the current study, we consider the scientific aspects of the identification of studied plants, as well as questions on their legal status.

Methods and materials

The list of methods used in this work included analysis of different literature sources, scientific publications, laws, decree and legal acts directly or indirectly connected with the topic of research, studied object and its preservation on the state level. Samples collected on the territory of East Kazakhstan were used as the materials of the study.

Figure 1. Herbarium samples of *Amygdalus ledebouriana* Schlecht.
(IPBB) transferred to the herbarium fund in the Institute of Botany and Phytointroduction (Almaty)

After the survey of Naryn ridge (South Altai), geographical locations of *A. ledebouriana* populations were determined. The largest population was found in Katon-Karagai region near Kokterek village. Plants of *A. ledebouriana* were detected in northeastern bush slope of Sary-Shoky Mountain, Naryn ridge, South Altai, close to Kokterek village. GPS coordinates: N: 49º05', E: 84º29', 724 m above sea level. Almond plants were relatively large, 150–170 cm height with branched shoots. Samples were transferred in 2019 to the Institute of Botany and Phytointroduction for the verification of species, digitization and further storage in herbarium fund [9].

Results and discussion

Analysis of literature sources and herbarium samples had showed the presence of contradictions in the identification of two species, which had interfered species determination. Short comparative analysis of literature sources is presented in Table 1.

Table 1

Mentions of two representatives of dwarf almond section in historical sources

№	Sources	*A. ledebouriana* (*rus.* Mindal ledebura)	*A. nana* (*rus.* Mindal nizkiy)	Notes
1	2	3	4	5
1	*Lat*. Species plantarum. Carl Linnaeus, 1753.	Absent	*Amygdalus indica nana*	The *Amygdalus* genus and some of its representatives were described for the first time
2	*Ger*. Abhandlungen der naturforschenden gesellschaft zu Halle 2. Schlecht., 1854	*Amygdalus Ledebouriana*	*Amygdalus nana* L.	The species *Amygdalus Ledebouriana* was firstly described on Irtysh and Bukhtarma rivers. Close species: *Amygdalus nana*, *Altaica* Ledeb. From Altai flora. Differences in seeds shape. No illustrations

Continuation of Table 1

1	2	3	4	5
3	USSR Flora. Eds. Komarov V.A., Vol. 10, 1941	*A. ledebouriana* Schlecht. *(rus.* M. ledebura)	*A. nana* L. (*rus.* M. nizkiy)	*A. ledebouriana* have aslant-elongated on the base seed (Altai, Tarbagatai). No illustrations of *A. ledebouriana*. *A. nana* and *A. petunnikowii* L. are presented (Fig. 2).
4	Flora of Kazakhstan. Eds. Pavlov N.V., Vol. 4, 1961	*A. ledebouriana* Schlecht. (*rus.* M. Lebeburovskiy)	*A. nana* L. (*rus*. bobovnik; *kaz*. ishik-sabak)	*A. ledebouriana* have 7–9 mm calyx tube; higher, up to 2 m height bush. The base of seed is aslant-elongated. Distribution (endemic): 22 Altai, 23 Tarbagatai, 24 Dzungarian Alatau. Illustrations of close species (Fig. 2)
5	Illustrated identified of plant in Kazakhstan. Eds. Goloskokov V.P. Part 1, 1969	*A. ledebouriana* Schlecht. (*rus*. M. Lebeburovskiy)	*A. nana* L. (*rus*. bobovnik; *kaz*. ishik-sabak)	*A. ledebouriana* have 7–9 mm calyx tube; the base of seed is aslant elongated. Bush 1.5–2 m height. Flowering V-VI, ripening VI-VII. Grow on meadow and steppe mountain slopes in river valleys of Altai, Tarbagatai, Soongari Alatau. Endemic, ornamental plant. Illustrations of close species in Figure 2
6	Flora of China. Gu Kyingi and co-authors. Vol. 9, 1974, 1985 and 1986	Mentioned as synonym for *Amygdalus nana* L.	*Amygdalus nana* L., *Amygdalus ledebouriana Schlechtendal; Prunus nana* (Linnaeus) Stokes (1812), not Du Roi (1772); *P. tenella* Batsch.	Seed has oval or spherical shape, 1–2 (–2.5) × 1.2–1.8 (–2) cm, thick straw-yellow hairs; mesocarp is dry, split after ripening; endocarp is oval-spherical 0.8–1.8 (–2.2) × 1–1.5 (–1.7) cm. Thick curved ventral and dorsal area. Surface with irregular net-like shallow furrows. The base of seed is aslant with blunt apex. Illustrations of close species in Figure 2
7	Illustrated identified of plant in Russia. Gubanov I.A. with co-authors. Vol. 2, 2003	Absent in the list, but characteristic trait of *A. ledebouriana* "stone-fruit aslant-elongated" is used for *A. nana*	*Amygdalus nana* L.	Fruits are thick covered with yellow-gray hairs, round, slightly flattened. Seeds have irregular net-like shallow furrows with aslant-elongated base. Illustrations of close species in Figure 2
8	Plants of Kazakhstan. Traditional and scientific names. Arystangaliyev S.A. and Ramazanov E.R., 1977	*Amygdalus ledebouriana* L.	*Amygdalus nana* (*rus*. Mindal nizkiy, or bobovnik; *kaz.* Alasa badam)	The first published Kazakh names of two species. Contradiction in traditional names of *A. nana* (*kaz*. Alasa badam) with Flora of Kazakhstan (*kaz*. ishik-sabak) and Illustrated identified of plant in Kazakhstan (*kaz.* ishiksabak)
9	The list of vascular plants of Kazakhstan. Abdulina S.A., 1999	*A. ledebouriana* Schlecht.	*A. nana* L.	*A. nana* L. is marked with "?". Probably doubtful species
10	Decree of the Government of the Republic of Kazakhstan from 31.10.06 N 1034 "On Approving the Lists of Rare and Endangered Plant Species"	*Amygdalus ledebouriana*	Absent	Abstract name of the species without author's name and its synonymous
11	www.theplantlist.org	*Amygdalus ledebouriana* Schltdl. — synonym to *Prunus ledebouriana* (Schltdl.) YYYao	*Amygdalus nana* L. — synonym to *Prunus tenella* Batsch.	*Amygdalus* is synonym to *Prunus.* In other words, *Prunus* subgen. *Amygdalus* (L.) Focke, 1894

Illustrations of dwarf almonds representatives in various competent sources also have contradictions. Illustrations of almond seeds and fruits in various sources are highlighted in red. For example, in USSR Flora,

the shape of seed in the fruit of *A. nana* does not match with *A. nana* in Flora of Kazakhstan. In Flora of China (1986) this trait was ignored, and *A. ledebouriana* was named as *A. nana* (Fig. 2).

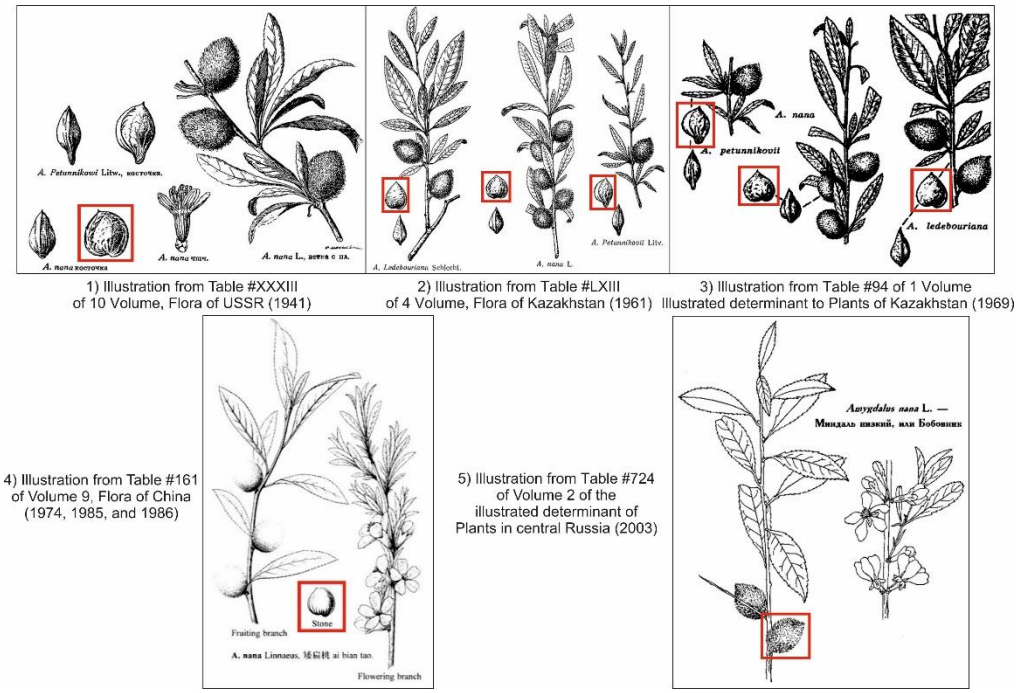

Figure 2. Illustration of dwarf almonds section representatives in various competent sources

Abovementioned contradictions between two species cast doubt on the presence of *A. nana* among the flora of East Kazakhstan, as well as jeopardize *A. ledebouriana*. According to the Law of the Republic of Kazakhstan dated July 7, 2006 No. 175 "On Specially Protected Natural Territories", *A. ledebouriana*, if present on specially protected natural territories, is an object of the state nature reserve fund of the Republic of Kazakhstan [10]. Species and subspecies mentioned in the list of objects in the state nature reserve fund are also listed in the Red Book of the Republic of Kazakhstan of different publication years. Abovementioned almond species can be considered within its distribution population or may be unique individual objects of the plant world with the unique scientific significance. According to the decree of the Government of the Republic of Kazakhstan from October 31, 2006 No. 1034 "On approval of the lists of rare and endangered plant and animal species", *A. ledebouriana* has a special status [11]. The extraction procedure is allowed only for propagation under specially created conditions, for scientific research and for breeding. Separate organisms (a whole plant), its various organs or derivatives (a substance that occurs in the process of biochemical reactions from another substance and, thus, is called derivative) can serve as an object for the extraction procedure.

According to the decision of the Council of the Eurasian Economic Commission from January 26, 2018 No. 15 "On approval of the rules of good practice for the cultivation, collection, processing and storage of raw plant originated materials", it is not allowed to collect and harvest raw materials from endangered medicinal plant species (*A. ledebouriana*) without the permission of the authorized bodies of member-countries in accordance with the provisions of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) [12].

According to Article No. 339 of the Criminal Code of the Republic of Kazakhstan from July 3, 2014 No. 226-V ZRK, illegal treatment of rare and endangered species, as well as species of plants or animals prohibited for usage, their parts or derivatives, provides various penalties depending on severity of the crime: from a fine of three thousand monthly calculation indices to imprisonment for 7–12 years with deprivation of the right to occupy certain positions or engage in certain activities up to five years with confiscation of property [13].

The development of criminal penalties or organization of events and measures to protect these plants are based on accurate species identification (according to the approved list of plants) of the plant biological objects (*A. ledebouriana*). During the classification, plant biological objects are assigned to the officially accepted taxonomic group of the current rank with accepted synonymic names in the state (Kazakh) and Russian

languages with the binary Latin nomenclature of the family, subfamily, genus, subgenus, section, species and subspecies of rare and endangered plants. Vegetative (leaves, stems, organs of the root system, etc.) and generative (inflorescences, sporangia, fruits, seeds, spores, etc.) organs, tissues, and cells serve as a biological object of plant origin. Also, this term includes various synthesized chemical and organic compounds, primary and secondary metabolites, etc.

The traditional procedure for identification of the sample under study is based on different apparent anatomical and morphological characteristics of species forming the keys for determination (described or implemented algorithm for the work with theses and antitheses). Usually, Flora of Kazakhstan [14], illustrated identifier of plants [15] and other scientific and methodological works are used as the main tool for determination.

These scientific and methodological works are unique multi-volume instruments for the study and identification of all plants growing on the territory of the Republic of Kazakhstan. Unfortunately, these works were published in Russian under the auspices of the Academy of Sciences of Kazakh SSR and are outdated at the moment. The information is not relevant for an accurate identification of species, which casts doubt on the procedure of traditional identification of rare and endangered plants growing on the territory of the Republic of Kazakhstan. names of taxonomic groups and their systematic structure do not correspond to international information databases of plants, such as World Flora Online [16], The Plant List [17], International Plant Names Index (IPNI), Royal Botanic Gardens Kew [18], and etc.

Official confirmation of the presence of a certain plant species is regulated by legal acts and its official and scientific public recognition without rebuttal. The main legal acts governing the recognition of a species and its scientific justification are listed in International Code of Nomenclature of algae, fungi, and plants. This code is a set of rules and recommendations governing the formation and use of the scientific names of plants, fungi and some other groups of organisms. The purpose of the code is to ensure that each taxonomic group has only one correct legal name that is used throughout the world. The remaining names are recognized as illegitimate and can be equated with synonyms or an erroneous definition (erroneous concept or outdated interpretation). Changes or additions to these taxonomic names is the main function of International Botanical Congress, which issues decisions of the plenary session on the basis of the resolution of the Congress' nomenclature section.

The absence of a constantly updated international system of taxonomic names for various groups of binary nomenclature in Kazakhstan complicates the international integration in the protection of rare and endangered plant species of Kazakhstan. A substantial and structural update of the approved list of rare and endangered plants taking into account the above-mentioned problems is required. It is also necessary to develop an updated electronic system (like Denali Flora and others) for identification of rare and endangered plants with different variations of anatomical and morphological characters depending on growing conditions. This system becomes popular in foreign countries, but, unfortunately, there is no analogue for identification of rare and endangered plants growing on the territory of the Republic of Kazakhstan.

Identification of biological plant objects, belonging to rare and endangered plants, is also based on the determination of its geographical distribution area. The distribution area of certain plant populations provides additional information for decision making. The geographical relief with various environmental factors and the availability of the necessary resources to support the vital functions of an organism serve to identify a limited distribution area. This information on the limited range is relevant for endemic plant species with a narrow ecological niche. Also, a large distribution area with the low density generates the presence of different ecotypes of one plant species with various anatomical and morphological characters in ontogenesis or vice versa. Unfortunately, in Kazakhstan, there are no information systems with specialized maps and updated information on the distribution of rare and endangered plants of national flora.

Another unique method for the identification of plant species' correspondence to the section of dwarf almonds in East Kazakhstan is barcoding based on the nucleotide sequence of certain sections of the plant genome. The use of DNA barcoding based on the identification of intra specific and inter specific polymorphism via DNA markers is a necessary tool for solving problems of identification and classification plant objects. Similar and different positions in nucleotide sequences are revealed in certain sections of the chloroplast and other plant genomes.

During the identification of samples, this method compares the obtained nucleotide sequences with an international database of various genomic parts of the plant. To improve this procedure, it is necessary to create a national database of complete genomes of rare and endangered plants based on all known markers. This scientific field actively develops on the basis of "Laboratory of molecular genetics" in Institute of Plant

Biology and Biotechnology belonging to the Committee of Sciences of the Ministry of Education and Science of the Republic of Kazakhstan (Almaty).

Exact classification and identification of the plant species is carried out by the competent authorities of the Center for Forensic Expertise under the Ministry of Justice of the Republic of Kazakhstan and its branches in various regions of our country. The type of accredited examination is No. 16.1 "Forensic Biological Expertise: Forensic Biological Investigation of Plant-Based Objects". However, the official list of this organization does not include a forensic botanical examination. As a separate type of this examination, biological research of plant objects is used in the Russian Federation and the countries of the European Union, only in specially accredited laboratories. Works on the study of the systematic and classification of rare and endangered plants is carried out by various methods on the basis of scientific centers, universities and institutes of the Ministry of Education and Science, the Ministry of Agriculture, the Ministry of Ecology, Geology and Natural Resources of the Republic of Kazakhstan.

Conclusion

It is necessary to develop new legal norms and special training programs for training specialists-researchers not only for the organization of environmental protection of natural resources and environmental management, the organization of specially protected natural territories of various significances, but also for supervisory bodies and internal affairs bodies in the field of environmental protection. Specialists of this group should be competent in the field of botany, geobotany, geography, ecology, and molecular genetics of rare, agricultural and medicinal plants. They also need to know the basics of forensic science and forensic examination of wild flora objects. The presence of these specialists with natural-scientific and legal competencies in the field of environmental protection in various organizations will increase the level of preservation of rare and endangered plants of the flora in East Kazakhstan.

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Шығыс Қазақстан флорасының ергежейлі бадамдар секциясының сирек кездесетін өкілдерін қорғаудың ғылыми және құқықтық аспектілері

Мақалада *Chamaeamygdalus* өкілдерінің (*Amygdalus ledebouriana* және *Amygdalus nana*) құқықтық мәртебесін анықтайтын және хронологиялық мәліметтері бар деректер көздері зерттелген. Мақалада әртүрлі әдебиет көздерінен және ғылыми мақалалардың иллюстрацияларынан алынған мәліметтерді талдау әдістері қолданылған. Қазақстанның ергежейлі бадамдар өкілдерін қорғаудың құқықтық аспектілері (мемлекеттік деңгейдегі қорғалуы үшін тікелей немесе жанама байланысы бар заңдар, жарлықтар және құқықтық актілері) алғаш рет зерттелген. Зерттеу нысаны үшін ботаника және құқық (экологиялық) саласындағы пәнаралық зерттеулердің бірігуі бұл жұмыстың өзіндік ерекшелігі болып табылады. Шығыс Қазақстан облысының аумағында жиналған биологиялық үлгілер және әртүрлі әдебиет көздерінен алынған деректер зерттелген. Зерттеу нәтижелері құзыретті әдебиеттер көздеріне сілтемелері бар салыстырмалы кесте және әртүрлі иллюстрацияларға ескертулері бар суреттер түрінде ұсынылған. Зерттеу нәтижелерін сирек кездесетін және жойылып бара жатқан *Amygdalus ledebouriana* Schlecht. және басқада осындай сирек өсімдіктер түрлерін сақтау бойынша шараларды ұйымдастыру барысында қолдануға болады. Зерттелген (ӨББИ) *A. ledebouriana* кеппешөп үлгілері Ботаника және фитожерсіндіру институтының кеппешөп қорына жіберілген (Алматы қ.).

Кілт сөздер: бадам, *Chamaeamygdalus, Amygdalus ledebouriana*, *Amygdalus nana*, өсімдіктерді қорғау, өсімдіктерді анықтау, сәйкестендіру, таксономия.

А.Е. Оразов, А.Б. Мырзагалиева, Г.М. Жангожина, Ш.Т. Тустубаева, А.С. Каратаева

Научные и правовые аспекты охраны редких представителей секции карликовых миндалей флоры Восточного Казахстана

В статье изучены материалы и различные источники на наличие хронологической информации представителей *Chamaeamygdalus (Amygdalus ledebouriana* и *Amygdalus nana)* и его правового статуса. Применены такие методы анализа информации в различных литературных источниках, как иллюстрации в научных статьях и др. Впервые изучены правовые аспекты охраны (законы, постановления и правовые акты, имеющие прямую или косвенную связь с темой исследования на предмет связи с объектом исследования и его защищенности на государственном уровне) представителей карликовых миндалей Казахстана. Оригинальностью данной работы является интеграция междисциплинарных исследований в сфере ботаники и права (экологического права) для изучения исследуемого объекта. В качестве материала для изучения были использованы образцы, собранные на территории ВКО, и различные источники. Результаты приведены в форме сравнительной таблицы и иллюстрации с примечаниями и ссылками на различные компетентные источники, которые могут быть использованы для видовой идентификации и организации мер по сохранению редкого вида растения *Amygdalus ledebouriana* Schlecht., а также для подготовки специалистов в сфере охраны окружающей среды Восточного Казахстана и для разработки новых правовых норм по сохранению редких и исчезающих видов растений. Исследуемые гербарные образцы *A. ledebouriana* (ИББР) были переданы в Гербарный фонд Института ботаники и фитоинтродукции (г. Алматы).

Ключевые слова: миндаль, *Chamaeamygdalus, Amygdalus ledebouriana, Amygdalus nana,* охрана растений, определение растений, идентификация, таксономия.

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Geoecological assessment of the dynamics of pollution of the Nura river and Samarkand reservoir

The ecological state of water bodies is formed as a result of the interaction of factors of self-purification. Anthropogenic load and is determined mainly by stationary and field studies. An actual task is to systematize theoretical and methodological approaches to the use of geo information technologies in the process of environmental research and the cartographic presentation of their results. The article discusses and analyzes the data of Kazhydromet for the period from 2008 to 2018, and assesses the quality of water by complete of hydro chemical indicators of four points. Chart diagrams were built on the analyzing data, priority substances and enterprises that make the greatest contribution to the pollution of the reservoir and river are identified.

Keywords: ecological cartography, surface water quality, pollutants, hydro chemical indicators.

Introduction

Indicators of the ecological state of water bodies include a significant number of hydro chemical and hydro biological characteristics. There are the numbers of water classifications by combining numerous physical, hydro chemical and biological characteristics. The possibilities of using complex classifications for cartographic purposes are in significant due to the high cost and laboriousness of relevant studies and, as a result, small number of definition points. For mapping purposes, the simplest indicators are needed, defined in as many points as possible and providing the ability to compare different water bodies. As such indicator, the most commonly used index of water pollution (WPI) [1].

The monitoring of surface water pollution is carried out using stationary posts. Water pollution, as well as atmospheric pollution, is a complex, multifactorial and highly dynamic process. The concentrations of various pollutants present in the aquatic environment are characterized by complex temporal dynamics and depending on the intensity of entry into water bodies, the rate of self-cleaning and sedimentation processes, the volume of the water mass, the nature and speed of its movement. Each of the listed pollution factors is relatively independent of the others and has its dynamics. Pollutant center water bodies with sewage from industrial and agricultural enterprises, public utilities, with surface run off due to flushing from contaminated areas, during precipitation from the atmosphere, from secondary chemical processes of the transformation of pollutants, from natural sources. Waste water volumes are determined by the process of their formation and accumulation in enterprises and everyday life. A feature of the pollution of water bodies is the sharp variability associated with the possibility of volley discharges from storage tanks, both technologically determined and emergency. Flushing from contaminated areas is also extremely uneven in time and occurs during rain water and melt water run-off, as well as during floods. Precipitation from the atmosphere is determined by the presence of precipitated (leaching) impurities in it and the presence of appropriate meteorological conditions. Precipitation from the atmosphere is determined by the presence of precipitated (leaching) impurities in it and the presence of appropriate meteorological conditions [2].

One of the major artificial reservoirs in the Central Kazakhstan is the Samarkand. Its construction began in 1934 and ended in 1940. Significant volumes of water from the Samarkand reservoir are used for their activities by the Arcelor Mittal Temirtau Metallurgical Plant JSC, Bassel Group LLP, Temirtau Electrometallurgical Plant JSC, Aktau Cement Plant, Aktau village, take water for irrigation horticultural partnerships. Located on the Nura river, the Samarkand reservoir is the center of large enterprises in the chemical, metallurgical and coal industries, which are distinguished by high water consumption and there lease of waste into the environment. In the river basin, Nura has many environmental issues. With the development of industry in the 20th century, water intake for industrial needs and the discharge of industrial waste into rivers began in the basin. Because of this, now the Nura river and its tributaries are contaminated with mercury compounds and oil products [3].

It should be noted that the quality of surface waters is determined by hydrochemical and hydrobiological indicators. Similar studies in the Central Kazakhstan will be used to analyze the state of surface waters of the Nura River basin. These studies are carried out by a branch of RSE Kazhydromet for the Department of Environmental Monitoring.

Now GIS technology is the main method of aggregation and visualization of geospatial data, as well as the technical basis of thematic maps based on them. Since data on environmental pollution are often very voluminous (for example, the results of long-term results of environmental monitoring) their analysis and mapping without the use of technical means of data bases and geodata banks and GIS are difficult. Environmental pollution is usually defined as the introduction into the environment or the appearance in it of characteristic physical, chemical or biological agents, or as an excess of the natural long-term average concentration of agents in the period under consideration.

An urgent task is the systematization of theoretical and methodological approaches to the use of geographic information technologies in the process of environmental research and the cartographic presentation of their results. The article presents a solution to this scientific problem. The purpose of environmental mapping is to analyze the environmental situation and its dynamics, i.e. identification of spatial and temporal variability of environmental factors affecting human health and the state of ecosystems. To achieve this goal, it is necessary to collect, analyze, evaluate, integrate, territorial interpretation and create a geographically correct cartographic representation of a very diverse, often difficult to compare environmental information [4]. The largest negative impact on the condition and quality of water in the Nura River is exerted by the combined waste water discharge channel of Arcelor Mittal Temirtau JSC and the Chemical and Metallurgical plant Temirtau Electro-Metallurgical Plant LLP. The main pollutants in this section of the river are nitrite nitrogen, copper, zinc, phenol, sulfates and petroleum products, several times higher than the maximum permissible concentrations. The purpose of the study was to identify the dynamics of changes in the water quality of the Samarkand reservoir and the Nura River in the period from 2008 to 2018.

Research and methodology

The initial information for mapping was data on the total emissions of pollutants into the water from stationary sources, the number of these sources for 2008–2018, according to the data of the State Hydrometeorological Service Kazhydromet. Based on this information, ananalysis was made of the state of the surface water of the Samarkand reservoir and the Nura river of the Central Kazakhstan and map diagrams of data for 2008 to 2018 from 4 posts were compiled:

1. Samarkand reservoir, 7 km above the dam of Temirtau (Karaganda);

2. Samarkand reservoir, 0.5 km along the alignment from the southern coast of the river. Within the city of Temirtau (Karaganda);

3. Nura River, 1 km above the combined waste water discharge of Arcelor Mittal Temirtau JSC and the Chemical and Metallurgical plant Temirtau Electro-Metallurgical Plant LLP, Temirtau (Karaganda);

4. Nura River, 1 km above the combined waste water discharge of Arcelor Mittal Temirtau JSC and the Chemical and Metallurgical plant Temirtau Electro-Metallurgical Plant LLP Temirtau (Karaganda) [5].

One of the most advanced mapping programs at the moment is Arc GIS, along with it, SAS. Planet is used to obtain satellite images with reference to the coordinate system. Using the SAS Planet program, you can create high-quality foundation for a future map; using the Arc GIS software modules, the map itself is created. Data on pollutants were placed in the database. This GIS has ample opportunities for working with internal and external databases, and in particular, it implements the standard Pyton language. Python extends to the entire Arc GIS system, turning into a language for analysis, data transformation, automation of cartographic processes and allows to increase the productivity of these works.

To work with spatial data, the Arc GIS10.1 software package was used. To obtain satellite images in conjunction with the coordinate system, the SAS. Planet program was used.

To determine the assessment of the temporary state of water in the Nura River and the Samarkand reservoir, one of the methods for assessing the quality of water bodies using a complex of hydrochemical indicators is used-the hydrochemical index of water pollution (Fig. 1). This index represents the average proportion of excess TLV for a strictly limited number of limited ingredients [6].

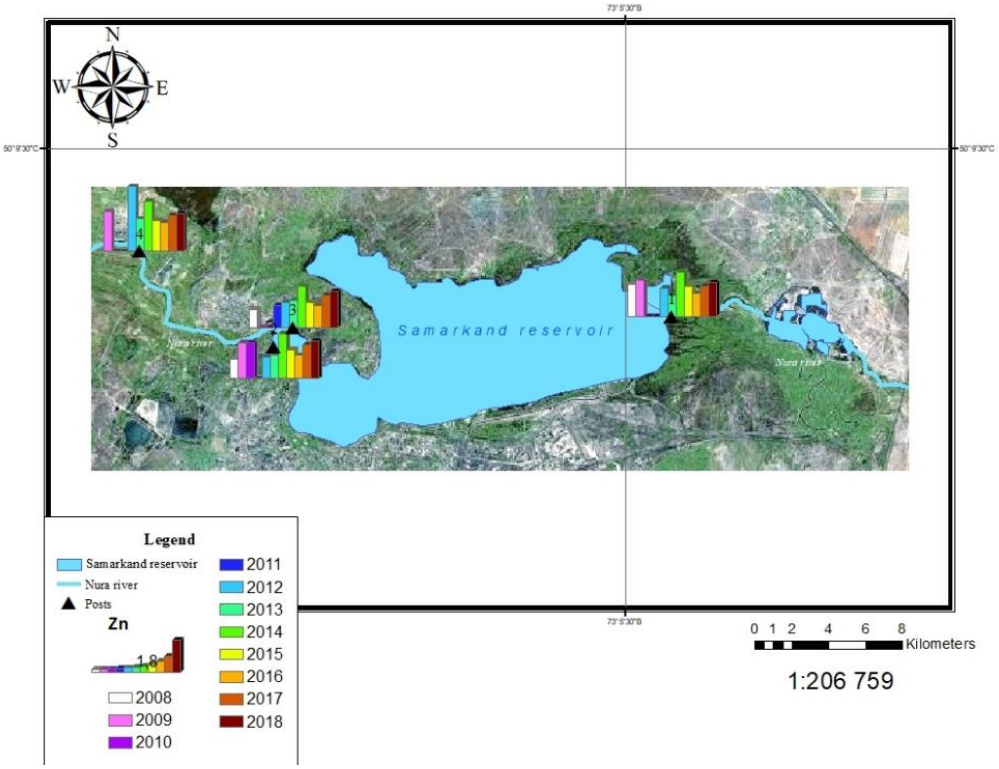

Figure 1. Diagram map of zinc emissions (Zn) in the Samarkand reservoir and the Nura river

Research results and discussion

Cartographic diagrams were compiled on the dynamics of pollution of individual elements. According to the data analysis of the RSE "Kazhydromet" in the upper part of the object, the zinc content varies from 0.012 mg/dm^3 (2013) to 0.025 mg/dm^3 (2014) (Fig. 1), the average level is 0.0174. It exceeds the MPC by 1.7 times. Over the entire period under observation, the level of pollution increases from 1-point to 4-point. This is due to the number of industrial facilities using water is increasing. The pollution concentration decreased in 2013 to an average of 0.014 mg/dm^3, but by 2014 it increased to 0.025 mg/dm^3. The zinc concentration at the 4-point exceeds the annual average of the previous points, their difference is 0.01 mg/dm^3. At the same time, the sequential cumulative increase in the concentration of zinc during movement downs tream the Nura River is also preserved. For example, the indicators of the four points under consideration were as follows: 0.019–0.021–0.020–0.024 mg/dm^3.

The analysis of sulfate pollution data (Fig. 2) shows that since 2008, from 248 mg/dm^3 by 2018, it has decreased to 183 mg/dm^3 (1.8TLV). Against this background, a single excess of concentration in 2012 to 367 mg/dm^3 stands out, which amount to 3.6 TLV.

For the observation period under consideration, data are available from 2014 to 2018, the level of manganese pollution (Fig. 3) decreased at 1 point to 0.148 at 0.046, 2 points from 0.16 to 0.055, 3 points 0.162 to 0.62 and 4 points from 0.191 to 0.021 which is an average of 28.6 %.

The analysis of copper pollution (Fig. 4) shows a down ward trend at the first point: the average copper concentration decreased from 0.0031 in 2008 to 0.0019 in 2018; at the second point, there were no changes in the same period; at the third point, they changed from 0.0024 to 0.0025; at the fourth point, there is also a decrease from 0.0031 to 0.0024. As with other polluting agents, there is a gradual increase in concentration from 2012 to 2014.

The next chart diagram (Fig. 5) shows that the waters of the Samarkand reservoir and the Nura river above the sewage discharge channel throughout the entire period belong to the third class of water quality, that is, to "moderately polluted". Water in the waste water discharge area has long belonged to the fourth quality class, but in recent years water quality has improved.

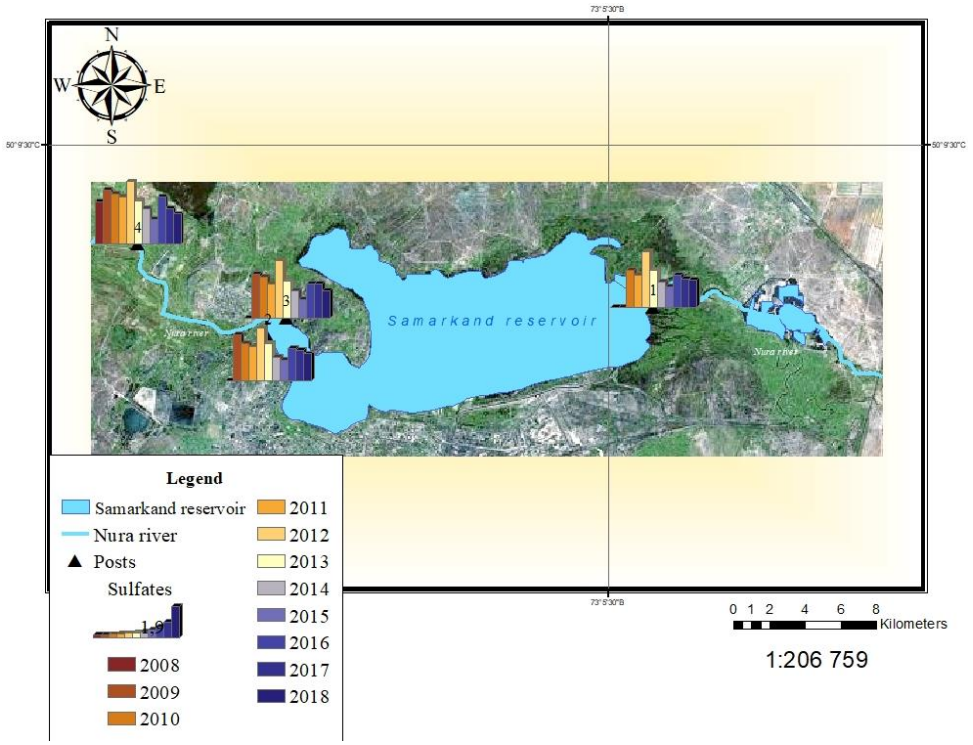

Figure 2. Diagram map of sulfate (SO_4) emissions into the Samarkand reservoir and the Nura river

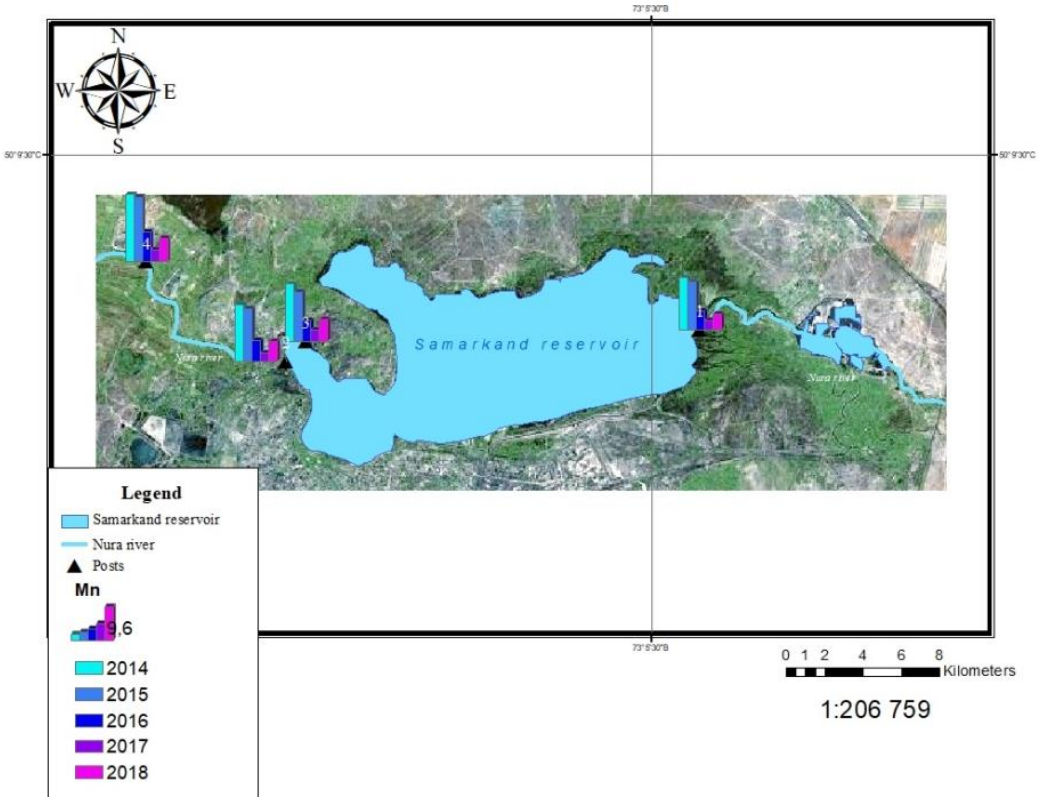

Figure 3. Diagram map of manganese (Mn) emissions into the Samarkand reservoir and the Nura river

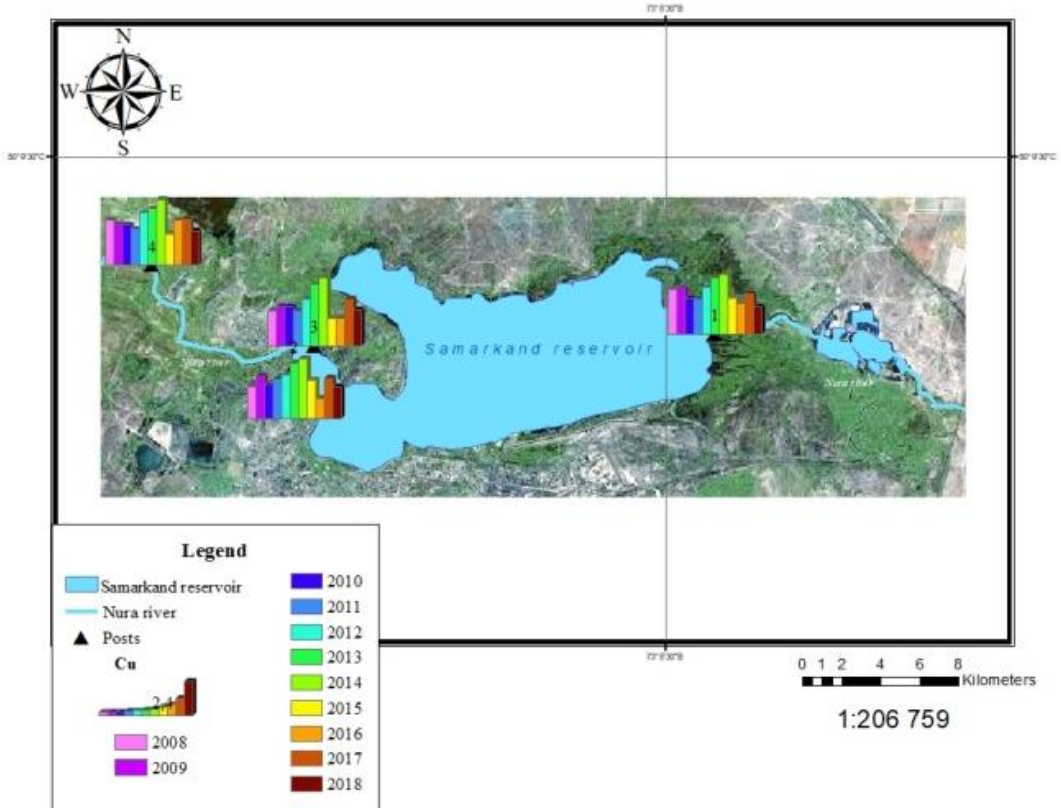

Figure 4. Diagram map of copper (Cu) emissions into the Samarkand reservoir and the Nura river

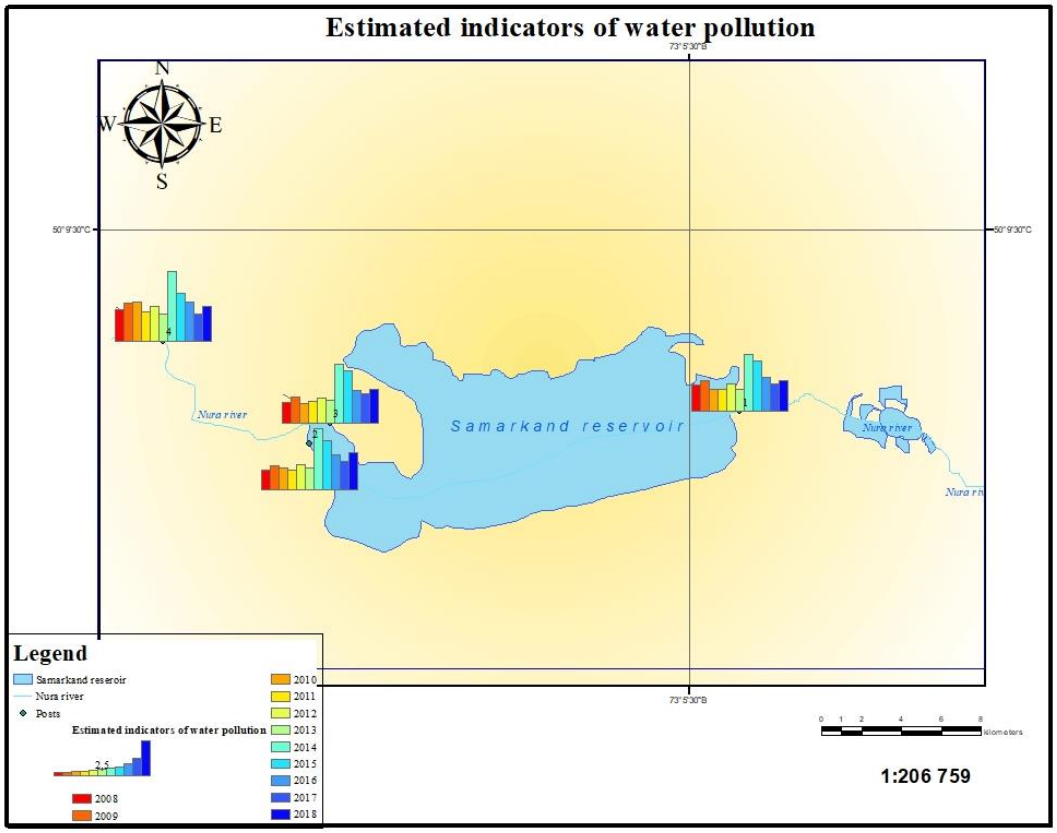

Figure 5. Diagram map of the water pollution index (WPI) of the Samarkand reservoir and the Nura River

The lowest WPI value is characteristic of the Nura River above the waste water discharge channel; a fairly low WPI value is also characteristic of the Samarkand reservoir. A significant polluting effect on the river is exerted by the channel of the combined discharge of waste water, both in the discharge area itself and downstream.

Using the example of surface water pollution for the period from 2008 to 2018, the WPI indicators were calculated.

Based on surface water pollution data, chart diagrams were compiled reflecting the dynamics over 10 years for common pollution since zinc, sulfates, copper and manganese, as well as for estimated water pollution index of water pollution index.

Conclusion

The state of surface water quality of the Samarkand reservoir shows an excess of the TLV for copper, zinc and manganese.

According to data from 2008 to 2018 water quality is estimated by a set of hydrochemical indicators of four points. As the results of the study showed, the average annual concentration of sulfates, manganese, copper, zinc has increased values at 2 and 4 points compared to1 and 3. They are characterized by maximum TLV values with a large spread in all indicators for the period under consideration. On the example of surface water pollution for the period from 2008 to 2018 using initial data, they prove that industrial facilities affect the quality of water in a reservoir and a river. As can be seen from the list, the greatest danger to the object under consideration is metal pollution. The main pollutants for these substances are Arcelor Mittal Temirtau JSC and the chemical and metallurgical plant Temirtau Electrometallurgical Plant JSC. As the calculations showed, the state of the quality of water resources can be attributed to the class of moderately polluted waters.

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Нұра өзені және Самарқанд су қоймасының ластану динамикасын геоэкологиялық бағалау

Су объектілерінің экологиялық жағдайы өзін-өзі тазарту факторларының өзара іс-қимылы нәтижесінде қалыптасады. Антропогендік жүктеме негізінен стационарлық және далалық зерттеулермен анықталады. Экологиялық зерттеулер барысында геоақпараттық технологияларды пайдаланудың теориялық-әдіснамалық тәсілдерін жүйелеу және олардың нәтижелерін картографиялық ұсыну өзекті болып табылады. Мақалада Қазгидрометтің 2008 жылдан бастап 2018 жылға дейінгі кезеңдегі деректері қарастырылған және талданған, сондай-ақ гидрохимиялық көрсеткіштердің жиынтығы бойынша судың сапасы төрт баллмен бағаланған. Алынған мәліметтерді талдау негізінде графикалық диаграммалар жасалған, су қоймасы мен өзеннің ластануына үлкен үлес қосатын басым заттар мен кәсіпорындар анықталған.

Кілт сөздер: экологиялық картография, жерүсті суларының сапасы, ластағыштар, гидрохимиялық көрсеткіштер.

А.А. Рахметова, Р.Р. Бейсенова, К.М. Акпамбетова

Геоэкологическая оценка динамики загрязнения реки Нуры и Самаркандского водохранилища

Экологическое состояние водоемов формируется в результате взаимодействия факторов самоочищения. Антропогенная нагрузка определяется в основном стационарными и полевыми исследованиями. Актуальной задачей являются систематизация теоретико-методологических подходов к использованию геоинформационных технологий в процессе экологических исследований и картографическое представление их результатов. В статье рассмотрены и проанализированы данные Казгидромета за период с 2008 по 2018 гг., а также оценено качество воды по совокупности гидрохимических показателей из четырех баллов. Диаграммы построены на основе данных анализа, определены приоритетные вещества и предприятия, которые причиняют наибольший вред в загрязнении водохранилища и реки.

Ключевые слова: экологическая картография, качество поверхностных вод, загрязнители, гидрохимические показатели.

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Анализ флоры травянистых растений города Алматы

В статье приведен таксономический, биоморфологический, географический анализ биоразнообразия флоры травянистых видов четырех районов г. Алматы, которая представлена 174 видами, которые относятся к 132 родам и 39 семействам, где двудольных растений насчитывается 149 видов (85,6 %), однодольных — 24 (13,7 %). Анализ крупнейших семейств флоры травянистых видов показал, что ведущими являются *Asteraceae* (37; 21,2 %), *Poaceae* (27; 15,5 %), *Brassicaceae* (15; 8,6 %), *Scrophulariaceae* (12; 6,9 %), *Lamiaceae* (9; 5,1 %), *Fabaceae* (8; 4,6 %), *Polygonaceae* (5; 2,8 %), *Malvaceae* (5; 2,8 %), *Ranunculaceae* (5; 2,8 %), *Rosaceae* (5; 2,8 %), содержащие в своем составе 128 видов, или 73,5 %. Богатыми по числу видов оказались роды: *Veronica* (11 видов; 6,3 %), *Artemisia* (5; 2.8 %). При анализе жизненных форм лидирующее положение групп травянистых поликарпиков обнаружено у 138 видов, или 79,3 %, среди которых господствует группа длиннокорневищных растений (42,0 %). Изучение географических элементов показало преобладание видов с широкими ареалами, где доминирующее положение занимают виды голарктической, палеарктической, космополитной, евразиатской, древнесредиземноморской, средиземноморской и горносреднеазиатской групп.

Ключевые слова: биоразнообразие, травянистая флора, город Алматы, сорные растения.

Введение

В последнее время влияние различных факторов человеческой деятельности на природу привело к необратимым изменениям и трансформациям флоры и растительности в крупных городах нашей страны, каким является г. Алматы. Планомерные работы по изучению флоры казахстанских городов начались лишь в последнее десятилетие XXI века [1].

В Алматы — городе республиканского значения Республики Казахстан, имеющем многолетнюю историю, прежде не проводилось целенаправленного изучения городской травянистой флоры.

В последнее десятилетие рост новостроек в черте города и его окрестностях усилил процессы антропогенного воздействия на урбанофлору. В связи с недостатком информации по травянистой флоре г. Алматы возникла необходимость её детального изучения с применением современных методик.

Город Алматы расположен у подножия гор Заилийского Алатау на крайнем юго-востоке республики с довольно мягким климатическим режимом. Климат Алматы континентальный и характеризуется влиянием горно-долинной циркуляции, что особенно проявляется в северной части города, расположенной непосредственно в зоне перехода горных склонов к равнине [2]. Структура почвенного покрова Алматы полностью определяется вертикальной зональностью Заилийского Алатау, где с изменением высоты меняются и природно-климатические зоны, и пояса, соответственно, и почвенно-растительный покров. Верхняя часть — урочище Медео расположено в лугово-лесостепной зоне с выщелоченными чернозёмами, тёмно-серыми лесостепными и горными лесолуговыми почвами. Ниже на высоте от 1000 до 1200–1400 м над у.м. расположена степная предгорная зона со следующими поясами (подзонами), это пояс высоких предгорий (прилавков) с чернозёмами и пояс предгорных тёмно-каштановых почв, которые начинаются от 750 до 1000 м [3]. Необходимо отметить, что изучение травянистых растений урбанизированных территорий осложняется тем, что почвы г. Алматы подвергались длительному антропогенному воздействию. Естественные почвенные горизонты в городах перекрыты привозными грунтами, изолированы от атмосферного воздуха различными твёрдыми покрытиями, такими как асфальт, бетон, брусчатка и т.п. Известно, что городские почвы поглощают химические загрязнители из воздуха. Темпы самоочищения почвы значительно ниже, чем у подвижных сред — воды и воздуха, и однократно попавшие в неё вещества могут наносить вред растениям в течение длительных периодов времени. Под влиянием выхлопных газов содержание свинца в травянистых растениях увеличивается в 50–100 раз [4].

Нами проведены исследования флоры травянистых растений г. Алматы, которая состоит из 8 районов (Медеуский, Бостандыкский, Турксибский, Алатауский, Жетысуский, Алмалинский, Ауезовский

и Наурызбайский) (рис. 1). Общая площадь 8 исследуемых районов г. Алматы составляет 529,52 км². Площадь Медеуского района равняется 99,4 кв. км, Бостандыкского района — 93,6, Алмалинского района — 18,2, Ауезовского района — 77,6, Жетысуского района — 34,5, Алатауского района — 75,76, Наурызбайского района — 69,76 и Турксибского района — 60,7 кв. км (табл. 1) [5].

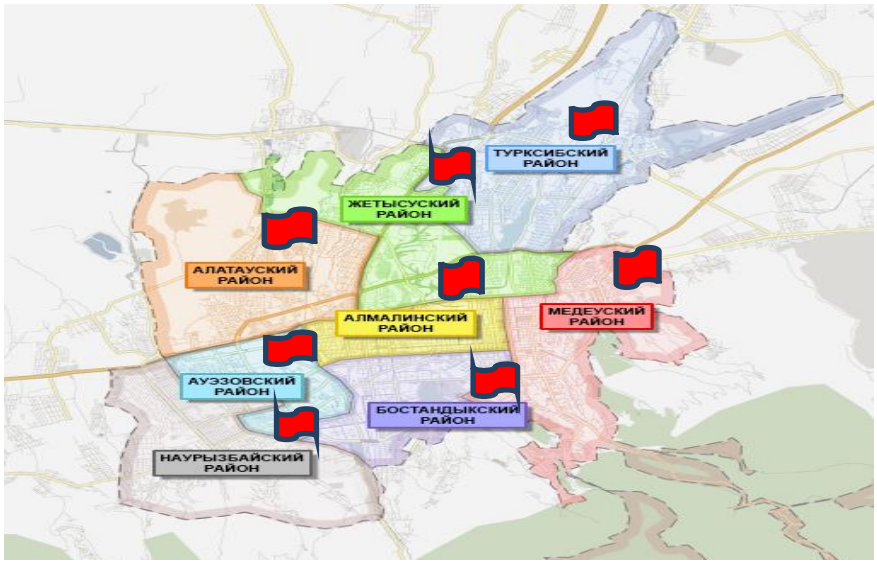

Рисунок 1. Схема-карта г. Алматы

Таблица 1

Общая характеристика исследуемых районов г. Алматы

Район	Дата основания	Площадь, км²	Численность населения, тыс. чел.	Природная зона
Алмалинский	1936	18,2	200,408	Степная
Жетысуский	1936	34,5	141,9	Степная
Медеуский	1936	99,4	181,085	Лесостепная
Турксибский	1938	60,7	188,437	Степная
Бостандыкский	1966	93,6	302 750	Степная
Ауезовский	1972	77,6	309,478	Степная
Алатауский	1993	75,76	158,300	Пустынно-степная
Наурызбайский	2014	69,76	157,0	Степная

Ниже приводится общее количество парков, скверов, бульваров, зеленых зон в г. Алматы (табл. 2).

Таблица 2

Общее количество парков, скверов, бульваров, зеленых зон в г. Алматы

Районы	Наименование					
	Парки	Рощи	Аллеи	Бульвары	Скверы	Зеленые зоны
Алмалинский	1	–	–	9	21	4
Ауезовский	1	–	–	1	6	15
Жетысуский	2	1	–	–	6	13
Алатауский	–	–	1		16	14
Бостандыкский	3	–	–	5	10	2
Медеуский	3	1	–	9	30	7
Наурызбайский	–	–	–	–	1	2
Турксибский	2	–	2	1	10	1
Всего	**12**	**2**	**3**	**25**	**100**	**58**

Материалы и методы исследования

Основными методами исследования городской флоры травянистых видов растений г. Алматы являлись общепринятые классические методики ботанических и флористических исследований: в полевых условиях использовался традиционный метод маршрутно-рекогносцировочный. Сбор и обработка гербарного материала проводились по общепринятой методике. Экземпляры древесных, кустарниковых и травянистых растений собирались в гербарные папки с описанием мест сбора (зафиксированные с помощью GPS), даты и коллектора. Сбор и обработка гербарного материала были проведены по общепринятой методике А.К. Скворцова [6]. В процессе определения гербария в качестве источников были использованы многотомные сводки: «Деревья и кустарники СССР» [7], «Флора Казахстана» [8], «Деревья и кустарники Казахстана» [9], «Растения Центральной Азии» [10], «Определитель растений Средней Азии» [11], «Иллюстрированный определитель растений Казахстана» [12]. Для уточнения видовых и родовых названий применялись последние сводки С.К. Черепанова [13], С.А. Абдулиной [14], А.Л. Тахтаджяна [15]. Типы ареалов исследуемых видов растений выделены нами согласно классификациям, разработанным Е.М. Лавренко [16], А.И. Толмачевым [17], Р.В. Камелиным [18] и В.П. Голоскоковым [19].

Результаты исследования и их обсуждение

В результате проведенных нами исследований в течение 2015–2019 гг. на территориях 8 районов г. Алматы были обнаружены 174 травянистых растений, относящихся к 132 родам и 39 семействам.

Анализ таксономической структуры флоры травянистых видов растений в 8 районах г. Алматы показал отсутствие плауновидных растений и слабую представленность сосудистых споровых хвощей-папоротников — *Dryopteris filix-mas* (L.) Schott. Основу флоры травянистых видов растений, как видно из рисунка 2, составляют *Magnoliophyta* (покрытосеменные), на долю которого приходится 86,2 %, и совсем ничтожный процент к *Polypodiophyta* (папоротники), всего 1 вид, или 0,5 %. Класс *Liliopsida* (однодольные) представлен 3 семействами, или 7,7 % от общего количества семейств, 24 родами (18,8 %) и 24 видами (1,3 %), *Magnoliopsida* (двудольные) — 36 семейством (92,3 %), 107 родами (81,0 %) и 149 видами, или 85,6 % (рис. 2).

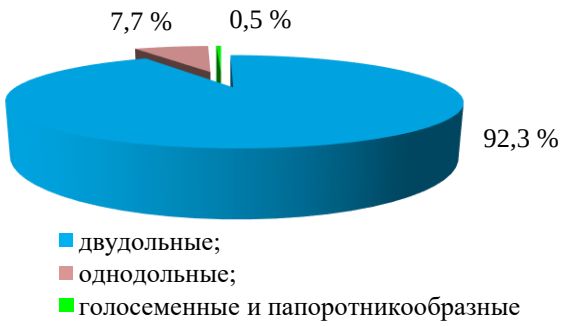

Рисунок 2. Общий состав флоры травянистых видов растений г. Алматы

Изучение крупнейших семейств флоры травянистых видов в 8 исследуемых районах г. Алматы показало, что ведущими по числу родов семействами оказались *Asteraceae* (37; 21,2 %), *Poaceae* (27; 15,5 %), *Brassicaceae* (15; 8,6 %), *Scrophulariaceae* (12; 6,9 %), *Lamiaceae* (9; 5,1 %), *Fabaceae* (8; 4,6 %), *Polygonaceae* (5; 2,8 %), *Malvaceae* (5; 2,8 %), *Ranunculaceae* (5; 2,8 %), *Rosaceae* (5; 2,8 %), содержащие в своем составе 128 видов, или 73,1 %, от всего состава флоры травянистых растений (табл. 3).

Остальные семейства содержат в своем составе от 4 до 1 вида. Так, семейство *Apiaceae* содержит 4 вида, или 2,3 %. Семейство *Chenopodiaceae* — 3 вида, или 1,7 %. Двенадцать семейств содержат в своем составе по 2 вида, или 1,1 %. К ним относятся следующие семейства: *Fumariaceae, Solanaceae, Plantaginaceae, Boraginaceae, Papaveraceae, Cannabaceae, Rubiaceae, Violaceae, Euphorbiaceae, Cuscutaceae, Urticaceae, Balsaminaceae*. И 15 семейств содержат в своем составе по 1 виду, что составляет 0,5 %. К ним относятся: *Primulaceae, Urticaceae, Paeoniaceae, Thymelaeaceae, Crassulaceae, Zygophyllaceae, Geraniaceae, Solanaceae, Convolvulaceae, Portulaceae, Caryophyllaceae, Amaranthaceae, Apocynaceae, Liliaceae, Cyperaceae* и *Dryopteridaceae*.

Таблица 3

Крупнейшие семейства травянистых видов, произрастающих в г. Алматы

Семейства	Количество родов, шт.	Количество видов, шт.	% от общего числа
Asteraceae	25	37	21,2
Poaceae	22	27	15,5
Brassicaceae	14	15	8,6
Scrophulariaceae	2	12	6,9
Lamiaceae	8	9	5,1
Fabaceae	6	8	4,6
Polygonaceae	4	5	2,8
Malvaceae	4	5	2,8
Ranunculaceae	4	5	2,8
Rosaceae	3	5	2,8
Всего	**92**	**128**	**73,1**

Анализ крупнейших родов травянистых видов растений г. Алматы показал, что по числу видов самым крупным родом оказался род *Veronica*, который содержит 11 видов, или 6,3 %. На втором месте расположился род *Artemisia* 5 видов (2,8 %). На третьем месте находятся роды: *Poa, Centaurea, Taraxacum, Potentilla* и *Centaurea*, содержащие по 3 вида каждый, что составляет 1,7 % от всего числа видов. 24 рода содержат в своем составе по 2 вида, что составляет 27,5 % от всего состава флоры травянистых растений (рис. 3). К ним относятся: *Bromus, Festuca, Euphorbia, Hyoscyamus, Agrostis, Hordeum, Arctium, Sonchus, Cirsium, Matricaria, Plantago, Mentha, Vicia, Trifolium, Impatiens, Viola, Brassica, Malva, Urtica, Chenopodium, Aquilegia, Polygonum.*

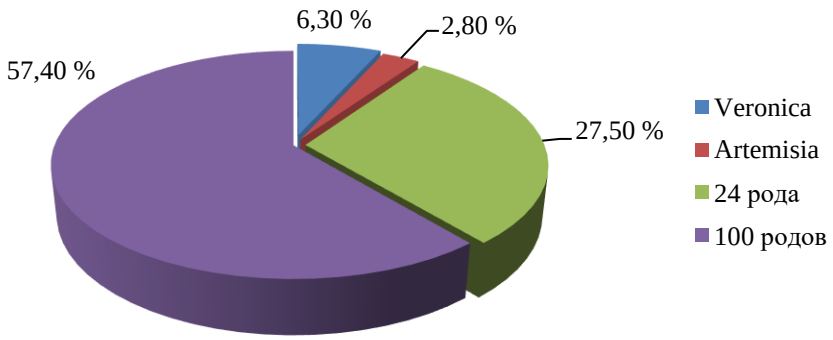

Рисунок 3. Соотношение ведущих родов травянистой флоры г. Алматы

100 родов содержат в своем составе по 1 виду, что составляет 57,4 % от всей флоры травянистых видов растений. К ним относятся: *Anizantha, Lolium, Echinochloa, Dactylus, Agropyrum, Bromopsis, Alopecurus, Eremopyrum, Avena, Elytrigia, Carum, Triticum, Phleum, Phragmites, Echium, Sorghum, Cynodon, Setaria, Carex, Gagea, Aegopodium, Daucus, Conium, Ambrosia, Ligularia, Tanacetum, Xanthium, Inula, Datura, Filago, Abutilon, Galinsoga, Carduus, Cichorium, Lactuca, Pyrethrum, Crepis, Achillea, Typha, Dryopteris, Onopordon, Apocynum, Rubia, Galium, Ranunculus, Ceratocephalus, Papaver, Chenopodium, Cardamine, Stellaria, Amaranthus, Atriplex, Fallopia, Persicaria, Rumex, Anagallis, Descurania, Erysimum, Berteroa, Cardaria, Thlaspi, Sisymbrium, Sinapis, Lepidium, Capsella* и др.

Изучение флоры травянистых видов не может быть полным без анализа жизненных форм, поскольку ее биоморфологическая структура отражает характер адаптации растений к набору условий среды, сложившихся в определенных экотопах. Поэтому ее изучение служит надежным инструментом познания экологии местообитания (табл. 4).

Таблица 4

Соотношение жизненных форм флоры травянистых видов растений г. Алматы по И.Г. Серебрякову (1962)

Жизненная форма	Количество видов	Процент от общего числа видов
1. Наземные травы		
1.1 Травянистые поликарпики	138	79,3
1.1.1 Стержнекорневые	25	14,3
2.1.1 Короткокорневищные	21	12,0
3.1.1 Длиннокорневищные	73	42,0
4.1.1Клубнеобразующие	3	1,7
5.1.1 Луковица	1	0,5
2 Травянистые монокарпики	51	29,3
2.1. Однолетники	40	23,0
2.2 Двулетники	11	6,3
Всего	**174**	**100**

Основой для анализа жизненных форм в наших исследованиях послужили системы жизненных форм И.Г. Серебрякова и К. Раункиера [20]. Необходимо отметить, что пространственное распределение экологических групп травянистых растений по отношению к условиям увлажнения в г. Алматы определяет небольшое разнообразие экотопов. В направлении от окраин к центру города, от зоны к зоне возрастает доля мезоксерофитов, а участие близких к ним групп прочих экоморф снижается, например, таких как ксеромезофиты и ксерофиты. Как известно, на урбанизированных территориях обеднение видового разнообразия идёт в основном за счёт выпадения растений естественной флоры. Так, далеко не все мезоксерофильные виды растений, даже при наличии оптимальных условий увлажнения, выдерживают загрязнённость среды, вытаптывание, высокую плотность почв, характерные для зон города.

Анализ жизненных форм по И.Г. Серебрякову показал, что во флоре травянистых видов растений г. Алматы лидирующее положение занимает группа травянистых поликарпиков (138 видов, или 79,3 %), среди которых господствует группа длиннокорневищных растений — 42,0 % (*Bromopsis inermis, Dactilys glomerata, Festuca pratensis* и др.), которые обладают максимальной способностью к вегетативному разрастанию и размножению и отличаются быстрым захватом территории. Другую группу составляют виды, неспособные к активному вегетативному размножению: стержнекорневые, короткокорневищные и клубнеобразующие — 49 видов (28,1 % от общего количества видов). Участие во флоре корнеклубневых растений незначительно. Среди них отмечаются в основном представители семейства *Fumariaceae.* Далее ведущее положение занимают травянистые монокарпики, которых насчитывается 51 вид (29,3 %), среди которых выделяются однолетники — 23,0 %. Группа монокарпиков встречается в семействах *Brassicaceae* (5 видов), *Poaceae* (7 видов), *Asteraceae* (8 видов), *Boraginaceae* (2 вида), *Malvaceae* (2 вида), *Balsaminaceae* (2 вида), *Chenopodiaceae* (2 вида), *Scrophulariaceae* (5 видов), *Caryophyllaceae* (2 вида), *Solanaceae (*2 вида), *Cuscutaceae* (2 вида) и др. Свой жизненный цикл они проходят за один или два года, завершая его цветением, плодоношением и отмиранием. Доля участия этой группы в составе флоры травянистых видов растений объясняется длительным и интенсивным антропогенным воздействием на растительный покров изучаемой территории. Как правило, это сорные растения. В нашем районе исследования широко распространены однолетние травы — *Capsella bursa-pastoris, Lepidium ruderale* и др. Мезоксерофиты являются наиболее многочисленной экологической группой во всех 8 районах города. В направлении от окраин к центру города их доля возрастает, так как к этой группе относится большинство неприхотливых сорных растений, не выпадающих из состава флоры с увеличением антропогенного влияния. Кроме того, в верхних зонах города абсолютно преобладают мезоморфные местообитания — частные дворы и огороды, клумбы, газоны, скверы и т.п. В городе основным источником заноса сорных видов является железнодорожный вокзал, для которого характерны своеобразные экологические условия.

На урбанизированных территориях всегда присутствуют сорные виды, число которых зависит от степени антропогенного воздействия на флору: чем больше нарушена флора, тем выше в ней процент сорных видов [21], что типично и для г. Алматы. Несмотря на снижение числа видов по направлению от окраин к центру города, доля сорных возрастает. Высокий процент сорных видов в г. Алматы

объясняется как практически отсутствием естественных местообитаний, так и немалым наличием частных домов с огородно-садовыми участками, на которых растут сорняки.

По продолжительности жизни сорные виды растений г. Алматы подразделяются на двулетники, однолетники и многолетники. Особенностями однолетних и двулетних сорных растений являются нетребовательность к антропогенным местообитаниям и быстрая приспосабливаемость к изменяемым условиям среды обитания.

Сорный элемент травянистой флоры г. Алматы представлен 68 видами (17,7 %), которые относятся к 54 родам и 20 семействам (табл. 5).

Таблица 5

Семейства сорных видов растений г. Алматы

Семейства	Количество родов, шт.	Количество видов, шт.	% от общего числа
Asteraceae	12	17	9,7
Poaceae	13	15	8,6
Brassicaceae	7	9	5,1
Fabaceae	2	3	1,7
Chenopodiaceae	2	3	1,7
Polygonaceae	2	2	1,1
Urticaceae	1	2	1,1
Balsaminaceae	1	2	1,1
Solanaceae	2	2	1,1
Plantaginaceae	1	2	1,1
Boraginaceae	2	2	1,1
Ranunculaceae	1	1	0,5
Papaveraceae	1	1	0,5
Portulacaceae	1	1	0,5
Amaranthaceae	1	1	0,5
Primulaceae	1	1	0,5
Malvaceae	1	1	0,5
Cannabaceae	1	1	0,5
Convolvulaceae	1	1	0,5
Apiaceae	1	1	0,5
Всего	**54**	**68**	**100**

Анализ ведущих семейств сорных видов в исследуемых районах г.Алматы показал, что самыми крупными семействами среди сорных растений являются *Asteraceae* (17; 4,3 %), *Poaceae* (15;3,7 %), *Brassicaceae* (2,2 %), *Fabaceae* (3; 0,75 %), *Chenopodiaceae* (3; 0,75 %). По 2 вида (0,5 %) содержат 6 семейств: *Polygonaceae, Urticaceae, Balsaminaceae, Solanaceae, Plantaginaceae, Boraginaceae.* И по 1 виду содержат 9 семейств, это *Ranunculaceae, Papaveraceae, Portulacaceae, Amaranthaceae, Primulaceae, Malvaceae, Cannabaceae, Convolvulaceae* и *Apiaceae*.

Среди сорных растений по степени натурализации преобладают виды, значительная часть которых разносится непреднамеренно и активно расселяется на нарушенных местообитаниях. Это широко распространённые сорные и рудеральные растения, такие как *Amaranthus retroflexus, Atriplex calotheca, Chenopodium album, Ch. hybridum, Sisymbrium loeseli, Plantago major, P. lanceolata, Cirsium arvense, Cichorium intybus, Bromus japonicus, Hyoscyamus pusillus, Ambrosia artemisiifolia, Thlaspi arvense, Datura stramonium, Abutilon theophrasti, Solanum nigrum, Cynodon dactylon, Urtica dioica, Capsella bursa-pastoris, Artemisia vulgaris, Artemisia annua, Artemisia absinthium, Arctium tomentosum, Eragrostis minor, Convolvulus arvensis, Xanthium strumarium* и др.

Выделение типов ареалов нами строилось на анализе современного распространения видов. Типы ареалов исследуемых видов растений выделены согласно классификациям, разработанным Е.М. Лавренко [16], А.И. Толмачевым [17], Р.В. Камелиным [18] и В.П. Голоскоковым [19]. Спектр географических элементов травянистой флоры г. Алматы показывает преобладание видов с широкими ареалами, где лидирующее положение занимают виды голарктической, палеарктической, космополитной, евразиатской, древнесредиземноморской, средиземноморской и горносреднеазиатской групп (табл. 6).

Таблица 6

Распределение видов травянистой флоры г. Алматы по типам ареалов в зависимости от географического происхождения

Типы ареалов	Количество видов, шт.	% от общего числа видов
Голарктический	45	25,8
Палеарктический	45	25,8
Космополитный	26	15,0
Горносреднеазиатско-иранский	14	8,0
Средиземноморский	9	5,1
Евразиатский	8	4,6
Древнесредиземноморский	7	4,0
Европейский	5	2,8
Тяньшано-памироалтайский	3	1,7
Тяньшанский	3	1,7
Среднеазиатский	2	1,1
Турано-иранский	2	1,1
Тарбагатае-северотяньшанский	1	0,5
Южносибирско-казахстанский	1	0,5
Евросибирско-казахстанский	1	0,5
Панноно-казахстанский	1	0,5
Североамериканский	1	0,5
Итого	**174**	**100**

Как показал географический анализ травянистой флоры 8 районов г. Алматы, растения голарктической (45 видов; 25,8 %), палеарктической (45; 25,8 %) и космополитной групп (26; 15,0 %) представлены в основном травянистыми растениями, к которым относятся большей частью сорные виды: *Ambrosia artemisiofolia, Tribulus terrestris, Conium maculatum, Veronica anagalloides, Sisymbrium loeselii, Xanthium strumarium, Descurainia Sophia, Datura stramonium, Stellaria media, Urtica dioica* и др. Средиземноморская (9; 5,1 %) и древнесредиземноморская (7; 4,0;) группы представлены 15 видами (8,6 %): *Brassica campestris, Cardaria draba, Lepidium ruderale, Lavatera thuringiaca, Abutilon theophrasti, Agrimonia asiatica, Geranium rectum, Impatiens brachycentra, Veronica cardiocarpa, Rubia tinctorum* и др.

Горносреднеазиатскоиранская (14; 8,0 %) и среднеазиатская (2; 1,1 %) группы представлены 6 видами, или 9,2 %, — *Aquilegia atrovinosa, Corydalis schanginii, Potentilla pedata, Populus tremula, Eremostachys speciose, Veronica persica, Rochelia leiocarpa, Taraxacum monochlamydeum* и др.

Евразиатская группа представлена 8 видами, или 4,6 %, это: *Ceratocephalus orthoceras, Trifolium repens, Potentilla erecta, Vicia tenuifolia* и др.

Европейская группа представлена 5 видами, или 2,8 %: *Brassica elongata, Asperugo procumbens, Cirsium arvense* и др.

Тяньшанская группа (12; 3,0 %) преставлена северотяньшанскими, тяньшано-памироалтайскими видами — *Viola acutifolia, Euphorbia lamprocarpa, Mentha interrupta, Taraxacum multiscaposum, Veronica cardiocarpa* и др.

Турано-иранская группа представлена 2 видами (1,1 %) *Papaver pavonium.*

Южносибирско-казахстанская, евросибирско-казахстанская, панноно-казахстанская группы представлены 3 видами, или 1,7 %, — *Gagea bulbifera, Cannabis sativa.*

Кавказо-крымская группа включает в себя всего 2 вида, или 0,5 %, — *Amelachier ovalis* и *Pinus pallasiana.*

Евросибирско-казахстанская группа представлена 6 видами, или 1,5 %: *Caragana arborescens, Rubus idaeus, Euphorbia virgata, Humulus lupulus, Cannabis sativa, Cuscuta europaea* и др.

Североамериканская группа представлена всего одним видом — *Galinsonga parviflora*.

Заключение

В результате исследований, проведенных на территории г. Алматы, было выявлено 174 вида травянистых растений, относящихся к 132 родам и 39 семействам. Таксономический анализ флоры травянистых видов растений характеризуется высоким процентом небольшого числа семейств, где на долю

первых десяти приходится 73,5 %. Низкая родовая и видовая насыщенность семейств травянистой флоры г. Алматы является показателем антропогенной нарушенности исследуемой территории.

Анализ сорной растительности травянистой флоры г. Алматы показал присутствие значительного количества сорных элементов (17,7 %), которые, в свою очередь, ведут к обеднению видового разнообразия флоры исследуемой территории.

Географический анализ флоры травянистых видов растений выявил, что значительную роль играют флористические элементы с ареалами бореального типа: горносреднеазиатского, горноцентральноазиатского, палеарктического и голарктического, составляющие 92,6 % от их общего числа.

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Г.А. Садырова

Алматы қаласының шөпті өсімдіктер флорасын талдау

Мақалада Алматы қаласының 4 ауданындағы шөптік өсімдіктер түрлері флорасының биоәртүрлілігіне таксономиялық, биоморфологиялық, географиялық талдау келтірілген. Онда 132 тұқымдасқа және 39 отбасына жататын 174 түрден тұратын, қосжапырақты өсімдіктердің 149 түрі (85,6 %), біржапырақты өсімдіктердің 24 түрі (13,7 %) ұсынылған. Шөп түрлерінің ірі тұқымдастарын талдау жетекші болып табылатындығын көрсетті: *Asteraceae* (37; 21,2 %), *Poaceae* (27; 15,5 %), *Brassicaceae* (15; 8,6 %), *Scrophulariaceae* (12; 6,9 %), *Lamiaceae* (9; 5,1 %), *Fabaceae* (8; 4,6 %), *Polygonaceae* (5; 2,8 %), *Malvaceae* (5; 2,8 %), *Ranunculaceae* (5; 2,8 %), *Rosaceae* (5; 2,8 %) құрамында 128 түрі бар немесе 73,5 %. *Veronica* (11 түрі; 6,3 %), *Artemisia* (5; 2.8 %) түрлердің саны бойынша бай. Өмірлік формаларды талдау шөпті поликарпиктер тобының (138 түр, 79,3 %) жетекші орнын көрсетті, олардың ішінде ұзынтамырлы өсімдіктерінің тобы басым болды — 42,0 %. Географиялық элементтерді талдау голарктикалық, палеарктикалық, космополиттік, еуразиялық, ежелгі Орта Жерорта теңізі, Жерорта теңізі және таукен ортаазиялық топтардың түрлері басым орын алатын кең таралу аймағы бар түрлердің басымдығын көрсетті.

Кілт сөздер: биоалуантүрлілік, шөпті өсімдіктер, Алматы қаласы, арамшөп өсімдіктер.

G.A. Sadyrova

Analysis of flora of herbal plants of the city of Almaty

The article provides a taxonomic, bio morphological, geographical analysis of the biodiversity of the flora of herbaceous plant species in 4 districts of the city of Almaty, which is represented by 174 species that belong to 132 genera and 39 families. Dicotyledonous plants have 149 species (85.6 %), monocotyledonous plants 24 species (13.7 %). The analysis of the largest families of flora of herbaceous species showed that the leading ones are: *Asteraceae* (37; 21,2 %), *Poaceae* (27; 15.5 %), *Brassicaceae* (15; 8,6 %), *Scrophulariaceae* (12; 6.9 %), *Lamiaceae* (9; 5.1 %), *Fabaceae* (8; 4.6 %), *Polygonaceae* (5; 2.8 %), *Malvaceae* (5; 2.8 %), *Ranunculaceae* (5; 2.8 %), *Rosaceae* (5; 2.8 %) containing 128 species or 73.5 % containing 128 species or 73.5 %. The genera was rich in the number of species: *Veronica* (11 видов; 6.3 %), *Artemisia* (5; 2.8 %). The analysis of life forms showed the leading position of groups of grassy polycarps (138 species, 79.3 %), among which the group of long-rhizome plants dominates — 42.0 %. An analysis of geographical elements showed the predominance of species with wide ranges, where the dominant position is occupied by species of the Holarctic, Palearctic, Cosmopolitan, Eurasian, Ancient Mediterranean, Mediterranean and Mountain Middle Asian groups.

Keywords: biodiversity, grassy flora, Almaty city, weed plants.

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Parameters of biochemical serum test of pigs in sarcosporidiosis

Sarcocystoses (sarcosporidioses, sarcocystoses) are invasive diseases caused by protozoa — coccidia of the genus Sarcocystis, the family Sarcocystidae. The main damage the disease causes livestock. Being localized in the muscles and heart of intermediate hosts (cattle and small cattle, pigs) cause exhaustion, anemia, decreased productivity and even death. In 1843, the German scientist Miescher first described characteristic intramuscular formations in the skeletal muscles of a domestic mouse, believing that these were clusters of parasites of unknown nature. Subsequently, similar formations in the muscles of other animals began to be described under different names: “Misher's bags”, “Rhine bodies”, “psorospermia” (ie scabies sperm). It was only in 1882 that Lankester began to call them meat cysts, or sarcocists, and proposed for their designation the corresponding generic name — Sarcocystis. A change in the level of iron, zinc, total and ionizing calcium in pig blood was observed during the clinical symptoms of sarcosporidiosis, when the disease is transmitted to the muscle. The article analyzes the changes in minerals and the activity of enzymes in the blood.

Keywords: sarkosporidiosis, hyperthermia, hypercupremia, hypercalcemia, enzymes, dynamics, sarcocystosis, blood serum.

Introduction

Porcine sarkosporidiosis causes serious economic damage to livestock. With sarcosporidiosis, the growth and development of piglets deteriorates, fertility decreases, the death of young animals is often observed, and meat products also worsen. To date, clinical manifestations and pathological changes have been described [1, 2], but the dynamics of biochemical parameters are not well understood.

The aim of our research is to study the changes in minerals in pig blood and the catalytic activity of enzymes in sarcosporidiosis.

Materials and method of study

The experiments were carried out in 2018 in a peasant farm in the Almaty region on 10 piglets of large white breed of 3–4 months of age, divided into 2 groups: 6 experimental animals were infested with *Sorcocystis suicanis* sporocysts at a dose of 75×10^3 parasites (I), 4 control ones did not infect (II). Keeping and feeding of the animals were the same. Blood was taken for examination before infection (2–4 times), then on 7, 14, 21, 30, and 40 days after infection. Biochemical studies included: determination of total calcium according to F. Umland, K.I. Meckenstok, ionized calcium — by calculation method according to I. Todorov, chlorides — by Levinson, copper — by I.W. Landers, V. Zak, iron — by W.T. Caraway, activity alkaline and acid phosphatases — by Bodansky, aspartic (AST) and alanine (ALT) transaminases — according to Reitman and Frenkel. The obtained data were statistically processed using the constant method [3].

Results and discussion

Data on the dynamics of mineralization in the blood serum of experimental invasive piglets with spores of sarcocystis suicanis are shown in Table 1. The severe period of the disease was characterized by an increase in the total calcium content and subsequently, a decrease in the transitional stage. The actual increase in the index compared to the initial data was observed on the 7th day (+69.1 %, P>98.8). Subsequently, the lowest levels of total and ionized calcium were recorded at 53.9 and –63.3 % (P<99.9), respectively, compared with data 30 days before infection [4, 5].

At the end of the experiment (40 days), the level of total calcium normalized, while ionized calcium decreased from the initial level by 61.9 percent (P>98.8). Serum iron and copper levels increased, and the maximum value was determined after 14 days, i.e. during clinical manifestations of the disease gradually decreases to 21–30 days by 111.0 and 62.4 % (P>99.9). During the parasitic period (40 days), the initial level of iron was recorded, and the copper content exceeded the initial level by 29.5 % (P>92).

Table 1

The dynamics of the content of minerals in the serum of the pancreas with sarcoporroidosis (M±m), n=10

Days of observation	Total calcium, mmol/L	Ionized calcium, mmol/L	Iron, mmol/L	Copper, mmol/L	Chlorides, mmol/L
Before infection	1.52±0.32 1.43±0.21	1.21 ±0.13 0.98±0.03	11.10±2.10 9.40±2.30	25.80±2.30 27.30±3.21	73.72±5.93 74.80±4.20
7 days after infection	2.57±0.10 l.84±0.18	1.28±0.06 1.00±0.12	17.40±2.70 11.8±1.40	32.10±2.70 28.0±2.10	86.85±3.88 80.0±1.24
14 days after infection	1.36±0.07 1.26±0.04	0.69±0.06 1.18±0.10	25.50±3.80 11.40±2.30	41.90±3.20 29.10±1.71	79.84±5.24 60.46±6.21
21 days after infection	1.01±0.07 1.18±0.23	0,61±0,07 1.23±0.21	21.70±2.62 9.50±2.41	27.40±2.20 31.0±2.31	75.42±4.34 84.20±3.25
30 days after infection	0.70±0.23 1.20±0.31	0.42±0.11 1.74±0.21	15.67±l.50 8.70±1.73	36.40±3.80 25.40±2.61	96.86±3.86 93.0±2.36
40 days after infection	1.24±0.09 1.17±0.33	0.46±0.05 1.70±0.14	10.00±2.20 8.7 ±2.32	33.40±2.80 30.10±2.36	75.69±1.13 64.2±2.35

Note. In the field of the test group, figures for the control group are indicated.

Changes in the amount of chloride in the blood serum in the experimental and control groups were not observed during the experiment, an average of 64.20±2.35 mmol/L.

The enzymatic activity of aspartic and alanine transaminases, alkaline and acid phosphatase in serum of pigs significantly changed with sarcosporidiosis (Table 2).

Table 2

Change in the activity of enzymes in the serum of pigs in experimental sarcosporidiosis (M ± m), n = 10

Days of observation	Aspartate aminotransferase, nmol/s. L	Alaninamino transferase, nmol/s. L	Alkaline phosphatase, nmol/s. L	Acidic phosphatase, nmol/s. L
Before infection	30.9±1.9 31.4±1.1	9,1±3.4 8,4±2.8	2.46±1.04 3.81 ±1.2	0.78±0.16 0.84±0.14
7 days after infection	25.5±3.8 31.3±06	14,2±3.1 6,2±1.7	11.19±1.84 4.04±2.1	0.59±0.19 0.81±0.22
14 days after infection	55.9±6.3 31.3±1.8	33.5±5.5 13.2±2.4	15.16±1.98 3.9±2.3	4.11±1.05 0.81±0.10
21 days after infection	59.3±2.5 32.5±2.1	17.8±2.9 10.2 ±1.5	16.61±2.67 2.7±1.7	1.64±0.17 1.7±0.12
30 days after infection	51.4±4.2 32.7±1.9	57.8±1.8 15.8±2.4	11.61±1.52 6.5±1.3	3.06±1.85 1.6±0.14
40 days after infection	40.2±3.8 26.0±4.1	29.2±6.04 19.1±2.8	9.03±1.52 5.5±1.2	1.38±0.33 1.21±0.32

Note. In the field of the test group, figures for the control group are indicated.

Thus, the actual growth of ACT and ALT — 67.6 and 268.1 % (P>99.9) was determined within 14 days, and the catalytic activity of alkaline and acid phosphatase in comparison with the initial data was 516.0 and 426, respectively, 0 % (all P>99.9). The highest activity of AKT and alkaline phosphatase on the 21st day was observed in the range from +91.6 to +575.2 % (P>99.9). High activity of these enzymes on the 40th day (+30.1 %, P>94); (+220.9 %, P>98); (+261.1 %, P>99) and acetic phosphatase remained in the original data. Actual changes in the control group of animals were not detected during the control.

Discussion of the study results

Thus, in a severe period of the disease, pancreatic disorders occur in the form of hyperkalemia, hyperbiliremia, hypercupremia. Detected hypercalcemia is a consequence of a kidney and urinary tract disorder; the decrease in the content of ionized calcium is due to a decrease in the biosynthesis of albumin proteins in serum, as well as a violation of the water balance and acid-base balance.

Hyperbiliremia can be associated with high hemolytic erythrocytes and, as a result, the growth of the gallbladder in the blood serum, hypercupremia, major muscle fibrosis due to liver damage, is the result of a high level of ceruloplasmin product. A sharp increases in the activity of transaminases. According to Y. Musil, the liver has deep lesions before necrosis [6]. Fluctuations in the chloride content in the experimental and control groups of animals were not observed.

Conclusions

Disruption in mineral metabolism in blood serum such as hyperdemia, hypercupremia, hypercalcemia, in the severe stage of sarcosporidiosis of the pig; an increase in the enzymatic activity of aspartic and alanine transaminases, alkaline and acid phosphatases is observed.

When the disease transitions into the chronic stage, the activity of AST, ALT, and alkaline phosphatase is maintained at a high level; the amount of ionized calcium is lower than the initial one; other indicators also are normalized.

A comparative study of the morphology of sarcocysts, cystozoites, and sporocysts under a light microscope of the sarcocysts we have identified from different mammalian species shows the presence of a wide variety of shapes and sizes of cysts, merozoites, and the structure of the cysts wall.

The size and shape of cysts depend largely on their age and degree of maturity.

The most stable diagnostic sign of Mature sarcocysts in different species of animals under a light microscope is the structure of the cystic wall and the size of Mature asexual stages of development of sarcocysts-merozoites.

The most stable diagnostic sign of mature sarcocysts in various animal species under a light microscope is the structure of the cyst wall and the size of the mature asexual stages of development of sarcocysts — merozoites.

According to the morphological characteristics of the cystic wall and Mature merozoites, in some cases, it is possible to distinguish species only with the help of an electron microscope.

When determining the type of sarcosporidium, the type of intermediate and definitive host is of great importance, along with the morphological and biological properties of the parasite.

Sarcosporidia, like other eimeriid spores, are parasites that are strictly specific to the intermediate host.

The dependence of the size of merozoites on the taxonomic affiliation of the final host was noted.

Analysis of morphological features of different types of sarcosporidia shows that the morphology of these parasites depends largely on the taxonomic affiliation of the final host.

In sarcosporidia, the final hosts of which are predatory mammals (Fox, Korsak, cat), merozoites are large, the wall of the cysts is thick with transverse striation, (under a light microscope).

The exception is sarcocysts from the house mouse, in which the wall of the cysts is smooth.

In sarcosporidia, the final hosts, which are birds of prey (Buzzard, owl, Kestrel), are merozoites smaller in size, the wall of the cysts is thin, smooth without transverse striation.

The selection of probable final hosts-predatory animals for setting up experiments to clarify the development cycle of detected sarcocysts is not an easy task, especially in wild animals.

Some help in selecting the likely final hosts of the sarcocysts under study can be provided by the regularities of the dependence of morphological features on the final host.

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Саркоспоридиоз кезінде шошқаның қан сарысуындағы биохимиялық көрсеткіштер

Саркоцистоздар (саркоспоридиоздар, sarcocystoses) — бұл кокцидиялардың Sarcocystis туысына, Sarcocystidae тұқымдасына жататын қарапайымдылар тудыратын инвазиялық аурулар. Негізгі зиянын мал шаруашылығына тигізеді. Аралық иелерінің бұлшық еттері мен жүрегінде (ірі және ұсақ малдарда, шошқада) оқшаулана отырып, малдың арықтауына, анемияға, өнімділіктің төмендеуіне және тіпті өлімге әкеледі. 1843 жылы неміс ғалымы Мишер (J.F. Miescher) үй тышқандарының (Mus musculus) бұлшық ет талшықтарынан саркоцисталарды тапты. Кейінірек осындай цисталар жануарлардың басқа да түрлерінің бұлшық еттерінен табылған. Олар әртүрлі атауларда сипаттала бастады: «мишер қапшықтары», «рейндік денелер», «псороспермалар» (яғни, қышыма спермалары). 1882 жылы Ланкестер шошқа саркоспоридиясын сипаттап, оларды Sarcocystis mischeri деп атады және осылайша Sarcocystis туысы атауы енгізілген. Саркоспоридиоз ауруының клиникалық белгілерін байқап, ауру бұлшық етке ауысқан кезеңде шошқа қанында темір, мырыш, жалпы және иониданған кальций мөлшерінде өзгерістер болатыны анықталған. Мақалада қандағы минералдық заттар мен ферменттердің белсенділіктерінің өзгерістері талданған.

Кілт сөздер: саркоспоридиоз, гиперсидеремия, гиперкупремия, гиперкальциемия, ферменттер, динамика, саркоцистоз, қан сарысуы.

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Биохимические показатели в сыворотке крови свиней при саркоспоридиозе

Саркоцистозы (саркоспоридиозы, sarcocystoses) — это инвазионные болезни, вызываемые простейшими — кокцидиями рода Sarcocystis, семейства Sarcocystidae. Основной урон болезнь наносит животноводству. Локализуясь в мышцах и сердце промежуточных хозяев (крупный и мелкий рогатый скот, свиньи), вызывают истощение, анемию, снижение продуктивности и даже падеж. В 1843 г. немецкий ученый Мишер (Miescher) впервые описал характерные внутримышечные образования в скелетной мускулатуре домашней мыши, полагая, что это были скопления паразитов неизвестной природы. Впоследствии сходные образования в мышцах других животных стали описываться под разными названиями: «мишеровы мешочки», «рейновские тела», «псороспермии» (т.е. чесоточные спермии). И лишь в 1882 г. Ланкестер стал называть их мясными цистами, или саркоцистами, и предложил для их обозначения соответствующее родовое название — Sarcocystis. Заметив клинические признаки заболевания саркоспоридиоза, он обнаружил, что в период перехода заболевания к мышцам в крови свиней происходит изменение содержания железа, цинка, общего и ионированного кальция. В статье проанализированы изменения активности минеральных веществ и ферментов в крови.

Ключевые слова: саркоспоридиоз, гиперсидеремия, гиперкупремия, гиперкальциемия, ферменты, динамика, саркоцистоз, сыворотка крови.

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The influence of atmospheric pollution on the growth and development of pine in the conditions of the Irtysh Semipalatinsk region

The wide range of common pine (*Pinus sylvestris* L.), its growth in different climatic zones and environmental conditions led to changes in morphological differences. The object of study is a ribbon woods population of pine (*Pinus sylvestris* L.) within the Irtysh region of the Semipalatinsk region. The article presents data on the growth dynamics of annual shoots of *Pinus sylvestris* in conditions of various gas contamination. The comparison of morphometric parameters of growth of pine shoots on three test areas, including the control area, was carried out. The pilot site was located within the city of Semey, the area of the Silicate plant (pilot site, Semey N 50.468442, E 80.212024) is considered the most polluted, two control areas were laid at a distance of 35 km (control area, Kashtak village, N 50.3983, E 80.6248) and 60 km from the city along the Borodulikha highway (control area, Borodulikha village, N 50.7336, E 80.8865). In an article describes the morphometric method of studying trees. For quantitative estimates of anthropogenic impact on the environment, data on the growth of woody plants are used, namely the study of the growth dynamics of annual shoots and pine needles. Based on this, 10 model pines were selected for the study at each site, aged 25–30 years, 6–9 meters high, d 24–36. The study of the length of the needles of experimental and control plants gave a clear characteristic of the changes arising under the influence of industrial pollution. As for the *Pinus sylvestris* growing in Priirtyshye, defined terms of the beginning, the termination, duration of visible gain.

Keywords: pine, shoots, trunk, skeletal roots, periodicity of growth, linear and radial growth, morphometric method.

Introduction

Emissions of harmful substances are concentrated mainly in industrial areas, where factories, transport, boilers are concentrated. Transport and thermal power plants account for a significant share of emissions in large cities. Emissions from industrial enterprises are one of the main causes of disruption of the sustainable functioning of ecosystems, including forests, causing their partial or complete degradation [1, 2].

In the new ecological situation plants play an important hygienic role, many plant species have the ability to extract from the atmosphere and accumulate various toxic gaseous compounds [1, 2].

The main object for assessing the state of forests in the Semipalatinsk region is the common pine (*Pinus silvestris* L). This is due to its wide range, as well as an important ecological role and economic importance. One of the most frequently used parameters for assessing the impact of industrial pollution, diagnostics of forest ecosystems is the morphometric method of the plant organism, since the morphological parameters of the plant are very sensitive to changes in growing conditions.

The degree of damage to species is directly dependent on the rate of absorption of gas and the intensity of gas exchange. Depending on the gas absorption, there are 2 types of damage, acute and chronic. Acute is noted with short-term exposure to plants of high concentrations of phytotoxicant, chronic — at low concentrations, but a long period of action [1, 2].

Pine is a qualitative bioindicator of the environment, so the choice of pine as a bioindicator is not accidental. To assess the state of atmospheric air for many years (2015–2019), we use the bioindicator pine (*Pinus sylvestris* L.). It is well known that it is a species that responds to pollution. This phytoindicator is widely distributed throughout the North-Eastern part of Kazakhstan, grows both on dry sands and in conditions of excessive humidity. In this regard, pine is a convenient object for bioindication of pollution levels in any area. Reactions of *Pinus sylvestris* L. to the presence of pollutants in the air reflect the overall level of environmental pollution with chemicals of various types. To assess the chemical load on the plant, its different characteristics are used. The morphological approach is the most common and easiest to implement [3]. In various literature sources as indicative signs it is recommended to use the value of the annual growth of the main shoot, the length of the leaf blade, the size of the generative organs [4]. Morphological and anatomical characteristics of

pine needles can also be used for indicative purposes. According to the authors, pine needles can be used as a bioaccumulator of aerogenic pollution [5, 6]. This is due to the fact that pine needles have the ability to effectively absorb pollutants, in particular, metal compounds, in the form of aerosols due to the diffusion deposition of the latter in the cavities and air channels of the leaf blade.

Recently, data on the growth of woody plants, namely, the study of the growth dynamics of annual shoots and pine needles, have been used more often for quantitative assessments of the anthropogenic environmental impact [7].

Materials and methodology

Our research was aimed at understanding the nature of the growth and development of pine (*Pinus sylvestris* L.) due to air pollution. In the spring of 2019, we began a study of the growth and development of Scots pine in the conditions of the Irtysh region of the Semipalatinsk region. The study was carried out at certain test sites (№ 1, 2, 3). Site № 1, the area of the Silicate Plant (experimental site, Semey N 50.468442, E 80.212024) is considered the most polluted. According to the data of the stationary observation network, the level of air pollution in the city is characterized as a high level of pollution, it is determined by the values of highest repeatability = 21 % (high level) and SI equal to 3 (high level) for phenol in the area of post № 4 (343 district, the area of the Silicate Plant 13/2) [8, 9].

Two areas (control site № 2, 3) were laid in a pine forest, they were located at a distance of 35 km (control plot, the area of the village of Kashtak, N 50.3983; E 80.6248) and 60 km from the sources of pollution along the Borodulikha highway (control plot, the area with Borodulikha, N 50.7336, E 80.8865). There are no large industrial facilities near the control plots, therefore, these research points were chosen. There is no data on air pollution in these areas.

For the study, we selected trees aged 25–30 years, height 6–9 meters, diameter 24–36 cm. The measurement was carried out with a measuring fork (pachymeter). The measuring fork serves to measure the diameters of the trunks of trees (Fig. 1) [10].

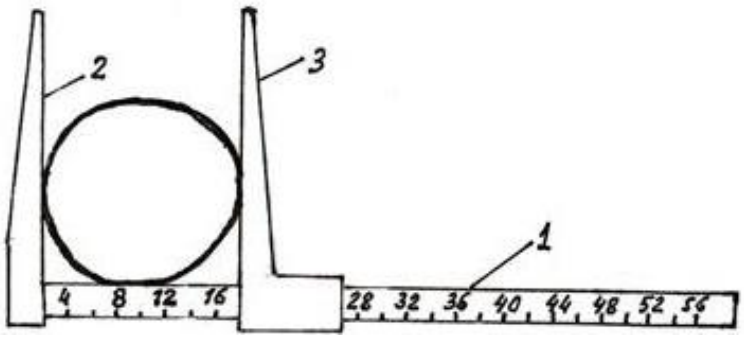

Figure 1. Measuring plug (pachymeter)

It can also be used to measure the height of trees (Fig. 2). At all sites (control and experimental), the studies were carried out on the same days.

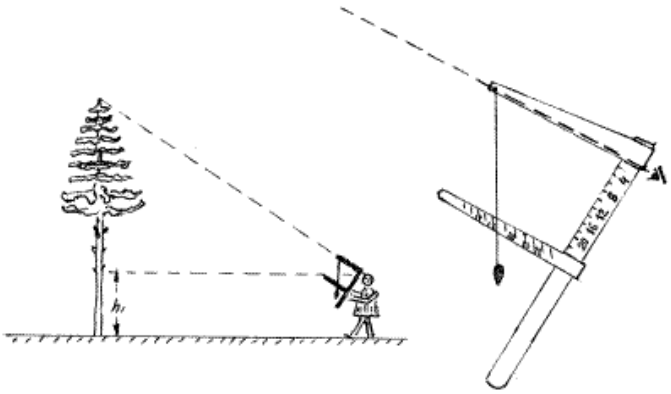

Figure 2. Measuring the height of a tree with a measuring fork

The influence of gas pollution on the growth and development of pine was studied by measuring the annual growth of shoots. For this purpose, took shoots in the middle part of the crown (repeatability 5 — fold, and in some cases 10 — fold).

Statistical data processing was performed using the Student's t-test calculation method [11].

Experiments and discussion

Morphological analysis

A comparative study of the annual growth showed that the value of the growth of shoots in experimental plants is less than in control plants (Table 1).

Table 1

Annual growth of shoots of common pine in the experimental and control sections of the pine forest of the Irtysh region of the Semipalatinsk region

№ site	Place of growth	Annual growth of shoots, cm				
		2015	2016	2017	2018	2019
№ 1 (experimental site)	The area of the Silicate Plant Semey N 50.468442, E 80.212024	9,7±0,8	9,36±0,65	7,6±0,1	7,6±0,3	7.3±0,21
№ 2 (control site)	The area of Borodulikha village N 50.7336, E 80.8865	11,5±0,12	10,8± 0,11	8,2±0,1	8,8±0,1	8±0,1
№ 3 (control site)	The area of the village of Kashtak N 50.3983, E 80.6248	11,5 ± 0,11	10,9±0,11	8,4±0,1	8,7±0,12	8,7±0,1

Among the control plants, the highest growth was found in model pine trees growing on the territory of the pine forest control area of the Borodulikha village, GPS coordinates: N 50.7336, E 80.8865 (plot number 2). Length of annual shoot growth of trees on this site in different years varies from 8 to 11.5 cm ($p < 0.05$) while the second control section, the area of the village Kashtak (section № 3), the GPS coordinates: N 50.3983, E 80.6248 shoot growth of trees from 8.4 to 11.5 cm ($p < 0.05$), and the smallest shoot growth shows experimental plot area Silicate plant, the GPS coordinates N 50.468442, E 80.212024 from 7.3 to 9.7 cm. According to the data, given in table 1, we can say that as the degree of anthropogenic load increases, the length of the shoot of pine decreases. Thus, from the data obtained, it can be determined that the ordinary pine has not completely lost its ability to respond to changes in external conditions, and also that the pine has morpho-metric limits of viability, which also depend on the external load.

Terms of growth of shoots and needles of Pinus sylvestris L.

The analysis of phenological data shows that under the influence of industrial pollution of the atmosphere, the annual growth of the pine tree is slowed down.

In the conditions of Semey of Irtysh, the growth of shoots of common pine begins on May, 10–12, and ends in mid-July. 10–15 days after the start of shoot growth, the beginning of the growth of needles is noted. The maximum duration of shoot growth is 64 days, needles 68 days.

A comparative study of the growth of shoots and needles by season showed that atmospheric pollution has a certain impact on the seasonal development of vegetative organs.

The growth of shoots in experimental plants began 5–6 days later compared to the control. In addition, we noted that the experimental plants are characterized by a reduction in the duration of growth of shoots and needles. So have pine, growing in city of (experienced) period growth 50 days, in the same time have in control sites 62–64 days.

When analyzing the phenological phase of pine, we noted that the experimental and control plants have different periods of development phases. In experimental plots the phenological stage occurs at 5–6 days later and ends by 8–9 days earlier.

The work of a number of researchers indicates the effect of gas contamination on the anatomical and morphological structure of leaves of woody plants [1, 2].

In this regard, we conducted a quantitative and qualitative study of the morphological and anatomical changes in the needles of common pine growing on the territory of the city of Semey (experimental plots № 1, Silicate Plant area) and pine forest (control plots № 2, Kashtak village, № 3, Borodulikha village).

Needles for study were selected from year to year from the middle part of the crown, from various branches. Studying the length of the needles of the experimental and control plants gives a rather distinct characteristic of the changes that occur under the influence of industrial pollution (Table 2).

Table 2

The growth dynamics of pine needles

№ site	Place of growth	Length of needles by years (cm)					Duration of preservation of needles (years)
		2015	2016	2017	2018	2019	
№ 1 (experimental site)	The area of the Silicate Plant Semey N 50.468442, E 80.212024	–	6,60±0,05	4,14±0,05	4,50±0,04	4,62±0,09	4
№ 2 (control site)	The area of Borodulikha village N 50.7336, E 80.8865	6,12±0,14	6,96±0,11	4,3±0,04	5,7±0,17	5,5±0,18	5
№ 3 (control site)	The area of the village of Kashtak N 50.3983, E 80.6248	6,06±0,19	6,22±0,13	6,72±0,6	6,02±0,14	5,20±0,09	5

A comparative study of the growth dynamics of pine needles showed that the sizes of experimental and control plants differ significantly. The length of the needles of the experimental trees in different years varies from 4.14 to 6.60 cm, while the trees of control area of the Borodulikha village have a length of needles from 4.3 to 6.96 cm ($p < 0.05$) and the second control section, the area of the village Kashtak have a length of needles from 5.20 to 6.72 cm ($p < 0.05$), although the climatic conditions in the growth zones of the control and experimental plants were the same.

Table 2 shows that the inhibition of the growth of needles is directly dependent on the habitat, that is the higher the air pollution, the shorter the length of needles. As for the regions under study as anthropogenic loads, it is possible to observe the duration of survival of needles on a pine tree. Since the needles of model pines were preserved on the experimental site only until 2016, needles were observed mainly in the trees until 2017. In the control areas, needles were preserved for up to 5 years. Some pines could be found needles in 2015. The fall of needles also shows the bio indication properties of pine.

Conclusions

Thus, a fatal decrease in the growth of shoots and needles in the conditions of the Semipalatinsk region is observed in 2015–2019.

An analysis of the presented indicators confirms the response of individuals of common pine to anthropogenic pollution: with an increase in the degree of anthropogenic load, the number of needles on the shoot decreases with a decrease in the length of the shoot itself and the length of the needles.

Based on these data, we can talk about the presence of morphometric limits of pine viability. Here, it is necessary to indicate that viability can be identified at different levels of the plant organism. However, we single out the morphological limit since the actual complex of physiological reactions of the plant usually finds its total expression in the actual growth rate, a certain orientation of which is ensured primarily by changing external conditions.

Thus, it can be seen from the obtained data that the annual growth of shoots and pine needles in the conditions of gas pollution in the city have not yet completely lost their ability to respond to changes in external conditions. Obviously, if we take the path of reducing harmful emissions, then a certain part of the pine stands can still be preserved. Conversely, if the level of pollution increases, urban pine plantations will die, certain sections of pine forests located near the city, within a radius of 20 km, may suffer, since the strongest impact of technogenic pollution on forest stands occurs on annual growth. Deterioration in the state of annual growth of shoot and pine needles is a sign of instability.

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Ертіс бойындағы Семей өңіріндегі кәдімгі қарағайдың өсуі мен дамуына атмосфералық ластанудың әсері

Кәдімгі қарағайдың (*Pinus sylvestris* L.) кең таралу аймағы, оның әртүрлі климаттық аймақтарда және экологиялық жағдайларда өсуі, оның морфологиялық айырмашылықтардағы өзгерістерге алып келді. Зерттеу объектісіне Ертіс бойындағы Семей өңіріндегі кәдімгі қарағайдың (*Pinus sylvestris* L.) тізбекті қарағай популяциясы алынған. Мақалада әртүрлі газдануы жағдайында өсетін кәдімгі қарағайдың жылдық өсу көрсеткіштерінің динамикасы туралы деректер келтірілген. Бақылау учаскесін қоса алғанда, үш сынақ алаңдарында қарағай өсімінің морфометриялық параметрлерін салыстыру жүргізілген. Тәжірибелік учаске Семей қаласының шегінде болды, Силикат зауытының ауданы (тәжірибелік учаске, Семей; N 50.468442, E 80.212024) барынша ластанған деп саналды, екі бақылау учаскесі 35 км (бақылау учаскесі, Каштак ауылы ауданы, N 50.3983, E 80.6248) және Бородулиха трассасы бойынша қаладан 60 км (бақылау учаскесі, Бородулиха ауылы ауданы, N 50.7336, E 80.8865) қашықтықта салынды. Жұмыста ағаштарды зерттеудің морфометриялық әдісінің сипаттамасы берілген. Қоршаған ортаға антропогендік әсерін сандық бағалау үшін ағаш өсімдіктерінің өсімі бойынша деректер пайдаланылды, атап айтқанда жылдық өсу мен кәдімгі қарағайдың қылқан жапырақтарының өсу серпінін зерттеу. Осыған байланысты зерттеу үшін әр учаскеде 25–30 жас аралығындағы, биіктігі 6–9 метр, d 24–36 модельді он қарағай таңдап алынды. Тәжірибелік және бақылау өсімдіктерінің қылқандарының ұзындығын зерттеу өнеркәсіптік ластанудың әсерінен пайда болатын өзгерістердің айқын сипаттамасын берді. Сондай-ақ, Ертіс өңірінде өсетін кәдімгі қарағай үшін өсудің басталу, аяқталу мерзімдері және көрінетін өсімнің ұзақтығы анықталды.

Кілт сөздер: кәдімгі қарағай, өсім, діңгек, өркен, өсу мерзімі, сызықтық және радиалды өсім, қылқан, морфометриялық әдіс.

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Влияние загрязнения атмосферы на рост и развитие сосны обыкновенной в условиях Прииртышья Семипалатинского региона

Широкий ареал сосны обыкновенной (*Pinus sylvestris* L.), произрастание её в разных климатических зонах и экологических условиях привели к изменениям в морфологических отличиях. Объектом изучения является ленточно-боровая популяция сосны обыкновенной *Pinus sylvestris* L. в пределах Прииртышья Семипалатинского региона. В статье приведены данные о динамике роста годичных побегов сосны обыкновенной в условиях различной загазованности воздуха. Проведено сравнение морфометрических параметров прироста побегов сосны на трех пробных площадях, включая контрольный участок. Опытный участок находился в черте города Семея, в районе Силикатного завода (опытный участок, Семей; N 50.468442, E 80.212024), который считается наиболее загрязненным; два контрольных участка были заложены на расстоянии 35 км (контрольный участок, район с. Каштак, N 50.3983, E 80.6248) и 60 км от города по Бородулихинской трассе (контрольный участок, район с. Бородулиха, N 50.7336, E 80.8865). В данной работе дано описание морфометрического метода исследования деревьев. Для

количественных оценок антропогенных воздействий на окружающую среду использованы данные по приросту древесных растений, а именно: изучение динамики роста годичных побегов и хвои сосны обыкновенной. Исходя из этого, для исследования были выбраны 10 модельных сосен на каждом участке, в возрасте 25–30 лет, высотой 6–9 м, диаметром 24–36 см. Изучение длины хвои опытных и контрольных растений дало отчетливую характеристику изменений, возникающих под влиянием промышленного загрязнения. Также для сосны обыкновенной, растущей в Прииртышье, определили сроки начала, окончания продолжительности видимого прироста.

Ключевые слова: сосна обыкновенная, побеги, ствол, скелетные корни, периодичность роста, линейный и радиальный прирост, морфометрический метод.

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Biodegradation and bioconversion of cellulose containing waste using bacterial and fungal consortium

In this paper, the results of study of the cellulose biodegradation using cellulolytic microorganisms are given. *Aspergillus awamori* VUDT-2, *Bacillus subtilis* 82 and consortium consisted of named strains were used in the enzymatic hydrolysis. Morphological features of these microorganisms were described using optical microscopy and by inoculation of suspensions on meat peptone agar. Initially, cellulose-degrading activity of microorganisms was tested in the medium containing reagent grade cellulose. The cultivation was carried out on a shaker-incubator in 750 mL Erlenmeyer flasks at a temperature of 30 °C and a stirring speed of 200 rpm for 6 days. As a result, the consortium showed better cellulolytic activity and 49.2 % of total cellulose was degraded in the end of the experiment. Then, the growth conditions of the consortium were optimized in terms of temperature, stirring speed and the initial pH of the medium. For further experiments, the dried distillers grains with solubles (DDGS) after alcohol production was used as a source of cellulose-containing waste. The content of the cellulose in the DDGS is 20.78 %. Under the conditions of temperature 30 °C and the stirring speed at 200 rpm the biodegradation level of cellulose using consortium of cellulolytic microorganisms was 70.24 % after 6 days.

Keywords: cellulose, bioconversion, biodegradation, reducing sugars, *Aspergillus awamori, Bacillus subtilis.*

Introduction

From the all biopolymers with carbohydrate structure, cellulose is the most common organic substance in the world. Different natural materials such as cotton and wood are mainly constituted by cellulose. Annually, there are more than 75 billion tonnes of this compound produced worldwide [1].

The applications of cellulose-based materials have been described in uncountable number of research papers in the fields of manufacturing new materials and nano-composites [2–4], biomedical [5, 6], environmental [7, 8], bio energy and bio ethanol production [9–11] and many other. Another application is that cellulose-containing materials can be used as a source for hydrolytic production of carbohydrates.

The molecular structure of cellulose is a linear polymer chain, consisted of an hydroglucose units which are linked with β-1,4-glucoside bonds. The number of monomeric units in the macromolecules of cellulose can be more than many thousands [12]. Nowadays, cellulose conversion to carbohydrates is mainly conducted using acid and enzymatic hydrolysis. Generally, acid hydrolysis is carried out using sulfuric acid or hydrochloric acid, while enzymatic hydrolysis is occurred by catalyzing with cellulolytic enzymes [13].

To this time, there are many microorganisms that have been described to catalyze the hydrolysis of polysaccharides. Namely, they are strains of *Bacillus pumilus, Pseudomonas sp., Trichoderma reesei, Trichoderma harzianum, Penicilliume chinulatum, Aspergillus phoenicis, Phanerochaete chrysosporium, Aspergillus niger* and other. All of them are considered as producers of cellulases [14].

Various types of cellulose-containing waste after pulp and paper industry as well as secondary products from sawmilling and wood processing, waste from agricultural crops and a countless number of wild plants can be used as a raw material for the hydrolytic production of carbohydrates. The cost, size of reserves and the possibility of their transportation to the area of hydrolytic production are the main criteria for the use of certain materials.

Thus, it should be noted that if even a small part of the listed sources is converted into useful products, it will give a very tangible and important renewable source of carbohydrates as well as starting compounds for microbiological synthesis.

At the present work, we carried out the enzymatic hydrolysis of cellulose using bacterial consortium, which is consisted of strains *Aspergillus awamori* VUDT-2 and *Bacillus subtilis* 82. The fraction of dried distiller grains with soluble (DDGS) is used as a source of cellulose-containing waste.

Materials and methods

The microbial cultures used in this study are *Aspergillus awamori* VUDT-2 and *Bacillus subtilis* 82, which are deposited in the official collection of the Branch of RSE "National Center for Biotechnology" under the Science Committee of Ministry of Education and Science of the Republic of Kazakhstan.

Bacillus subtilis 82 on Petri dishes with MPA forms rod-shaped colonies of white or yellowish color with a smooth or scalloped edge and a powdery surface. It is a gram-positive, aerobic bacterium, which is represented by single or associated cells. Spores are oval and located eccentrically, mostly closer to the center. According to the registration document, the optimum temperature for bacterial growth is 30 °C.

From morphological perspective, *Aspergillus awamori* VUDT-2 is a highly branched mycelium, divided by partitions. Conidia formed by micromycetes, covered with spikes. Conidia are round shape of brown color with a dark brown shade. On the medium with MPA, *Aspergillus awamori* VUDT-2 forms rounded colonies with a diameter of 18–20 mm with a smooth white edge and the surface is folded to the edges of the colony. The color is dark brown with an olive tint and exudate on the surface. According to the registration document, the optimum temperature for growth of *Aspergillus awamori* VUDT-2 is 30 ºC.

The samples of the cellulose-containing waste were obtained from malt distilling plant "Alfa Organic" LLP in Stepnogorsk. The content of cellulose in the DDGS is 20.78 %.

Pure cellulose and anthrone reagent were purchased from Sigma-Aldrich Co. Unless otherwise stated, all reagents were of analytical grade.

The pH was determined by the analyzer "Mettler Toledo Seven Multi S47-K".

The concentrations of cellulose and reducing sugars were determined by anthrone method. Firstly, the sample with cellulose was digested with concentrated sulfuric acid. Then, anthrone reagent was added and the green color mixture was measured spectrophotometrically on Bio Mate 3S (Thermo Scientific, USA) at wavelength λ = 620 nm [15].

Method of serial dilutions was used to count the number of the colony forming units.

Cell counting of the aerobic fungi *Aspergillus awamori* VUDT-2 was carried out by the method described in [16], an approximate method of serial dilutions with the calculation of the power of the number of microorganisms.

The morphological, cultural properties of microorganisms were studied by inoculating suspensions on MPA medium with incubation at 30 °C for 48 hours. Microscopic investigations were performed on an Olimpus 56BX microscope.

The following nutrient media were used in the experiments:

– Meat peptone broth (MPB), g/L: peptone — 10, glucose — 5, meat extract — 5, NaCl — 5, pH 7.2–7.4.

– Meat peptone agar (MPA), g/L: peptone — 10, glucose — 5, meat extract — 5, NaCl — 5, agar — 20, pH 7.4–7.6.

– Cellulose containing medium (CCM), g/L: cellulose — 100, peptone — 5, yeast extract — 2, $MgSO_4$ — 1, KH_2PO_4 — 1, pH 6.2.

– DDGS medium, g/L: DDGS —150, $MgSO_4$ — 1, KH_2PO_4 — 1, pH 6.

Results

Natural cellulose is an insoluble, semi crystalline polymer (Fig. 1). Polymer alignment formed its crystalline sections and they held together by hydrogen bonding and Van der Waals interactions [17].

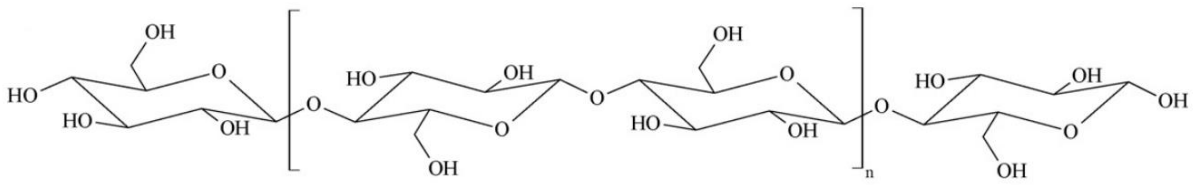

Figure 1. Chemical structure of cellulose

In nature, aerobic and anaerobic cellulolytic microorganisms degrade a major part of cellulose materials. Enzymatic degradation of cellulose occurs in several steps. Firstly, cellobiohydrolases acts on the reducing

and non-reducing ends and endoglucanases hydrolyze internal bonds, then b-glucosidase converts the cellobiose to glucose [18].

From the literature data [19, 20], both *Aspergillus awamori* and *Bacillus subtilis* are capable for producing cellulolytic enzymes.

At the present work, *Aspergillus awamori* VUDT-2 and *Bacillus subtilis* 82 were initially cultivated in the MPB medium. They were used to increase the reducing sugars because of their ability for bioconversion of cellulose. For this purpose, the inocula of *Aspergillus awamori* VUDT-2, *Bacillus subtilis* 82 and a consortium composed of strains of *Aspergillus awamori* VUDT-2 and *Bacillus subtilis* 82, were added in the amount of 10 % on the cellulose-containing medium.

The cultivation was carried out on a shaker-incubator in 750 mL Erlenmeyer flasks at a temperature of 30 °C and a stirring speed of 200 rpm for 8 days. The results of the experiment are shown in Figure 2.

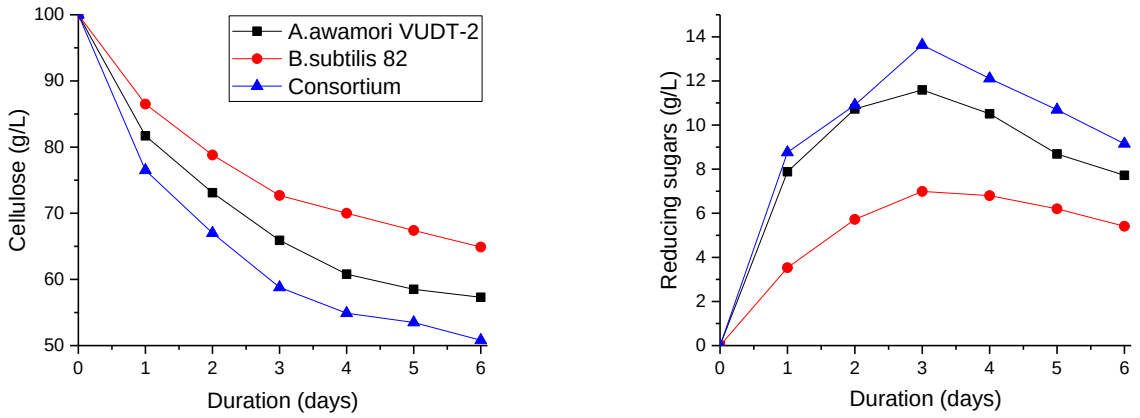

Figure 2. Bioconversion of cellulose to reducing sugars

During the experiment, in all cases, a decrease in the amount of cellulose and an increase in concentration of reducing substances were observed. This indicates the growth of *Aspergillus awamori* VUDT-2 and *Bacillus subtilis* 82. The number of microorganisms on the 6th day of the experiment increased to 10^9 CFU/ml and began to decrease on the 7th day of the experiment to 10^8 CFU/ml and on day 8 was 10^7 CFU/ml. Therefore, the optimal cultivation period was 6 days.

As it can be seen from Figure 2, in the case of the microorganism *Aspergillus awamori* VUDT-2, the cellulose content decreased to 58.0 g/L on day 6, and for the microorganism *Bacillus subtilis* 82, the decrease in cellulose content reached 65.5 g/l on day 6. When consortium was utilized, the cellulose content decreased to 50.8 g/L on day 6 and remained constant until the end of the experiment. The content of reducing sugars on the 3rd day increased and reached 7.0 g/l, 11.5 g/l and 13.63 g/l for *Aspergillus awamori* VUDT-2, *Bacillus subtilis* 82 and a consortium of microorganisms, respectively. However, during the experiment, the content of reducing substances was significantly reduced due to consumption by microorganisms, accordingly, the titer of cells decreased.

Therefore, the optimal option for biodegradation of cellulose is a consortium of microorganisms consisting of *Aspergillus awamori* VUDT-2 from *Bacillus subtilis* 82.

A study of the bioconversion of the cellulose-containing waste with a cellulose content of 20.78 % was conducted. For this, a consortium of microorganisms *Aspergillus awamori* VUDT-2 and *Bacillus subtilis* 82 was inoculated into medium with DDGS. The cultivation was carried out on a shaker incubator in 750 mL Erlenmeyer flasks at a temperature of 30 °C and a stirring speed of 200 rpm. The results of the experiment are presented in Figure 3.

According to the data presented in Figure 3, the cellulose content of the medium with DDGS decreased from 31.25 g/L to 9.3 g/L within 6 days. The content of reducing sugars increased in the first 3 days and reached 4.94 g/L, however, after that the content of reducing substances decreased due to the consumption of reducing sugars by microorganisms. On the 6^{th} day of the experiment, the cell titer was 10^8 CFU/mL. Further incubation led to a decrease in cell titer to 10^6 CFU/ml, which indicates a decrease in carbohydrate sources.

As a result of the bioconversion of a sample of cellulose-containing wastes using a consortium of cellulolytic microorganisms, the cellulose level decreased by 70.24 % over 6 days and did not change significantly on days 7 and 8.

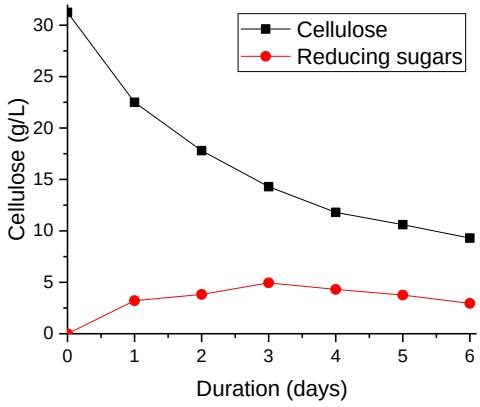

Figure 3. Bioconversion of cellulose-containing waste into reducing sugars using consortium of cellulolytic microorganisms

Thus, the consortium of microorganisms *Aspergillus awamori* VUDT-2 and *Bacillus subtilis* 82 is demonstrated high capability for the biodegradation of cellulose in waste from alcohol production.

Conclusion

As a result of growth of microorganisms *Aspergillus awamori* VUDT-2 and *Bacillus subtilis* 82, as well as a consortium on CCM, the cellulose content decreased to 58.0 g/L, 65.5 g/L and 50.8 g/L, respectively. The optimal incubation time is 6 days. Using a consortium of microorganisms *Aspergillus awamori* VUDT-2 and *Bacillus subtilis* 82, it was possible to lower the cellulose content in waste products of alcohol production to 70.24 % within 6 days. Furthermore, experiments will be conducted to reveal the mechanism of biodegradation and to study the cellulolytic enzymes of these strains.

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Бактериялар мен саңырауқұлақтардың консорциумын пайдалана отырып, құрамында целлюлоза бар қалдықтарды биодеградациялау және биоконсервациялау

Мақалада целлюлозолитикалық микроорганизмдерді пайдалана отырып, целлюлоза биодеградациясын зерттеу нәтижелері келтірілген. *Aspergillus awamori* VULT-2, *Bacillus subtilis* 82 және осы микроорганизмдерден тұратын консорциум көмегімен ферментативті гидролиз жүргізілген. Бұл микроорганизмдердің морфологиялық сипаттамасы микроскопия арқылы және ет-пептонды агарға себу жолымен сипатталған. Бастапқыда микроорганизмдердің целлюлозолитикалық белсенділігі құрамында аналитикалық целлюлоза бар ортада тестіленді. Өсіру 30 °С-да және араластыру жылдамдығы 200 айн/мин болғанда 6 күн бойы 750 мл Эрленмейер колбасының шейкер-инкубаторында жүргізілген. Нәтижесінде консорциум 49,2 % целлюлозаны ыдыратып, жақсы целлюлозолит белсенділігін көрсетті. Одан әрі эксперимент үшін құрамында целлюлозолитикалық қалдықтардың көзі ретінде спирттен кейінгі барданың қатты фракциясы бар ортада өсіру жүргізілген. Целлюлоза құрамы 20,78 % құрады. 30 °С-да және араластыру жылдамдығы 200 айн/мин болғанда целлюлозаның биодеградациясы 6 күн бойы целлюлозолитикалық микроорганизмдер консорциумын пайдалана отырып 70,24 % құрады.

Кілт сөздер: биоконсервация, қалпына келетін қанттар, *Aspergillus awamori, Bacillus subtilis.*

А.Е. Хасенова, Е.Н. Канафин, Н.К. Жаппар, В.М. Шайхутдинов, А.К. Шибаева

Биодеградация и биоконсервация целлюлозосодержащих отходов с использованием консорциума из бактерий и грибов

В статье приведены результаты исследования биодеградации целлюлозы с использованием целлюлозолитических микроорганизмов. Проведен ферментативный гидролиз с помощью *Aspergillus awamori* VUDT-2, *Bacillus subtilis* 82 и консорциума, состоящего из этих же микроорганизмов. Морфологическая характеристика данных микроорганизмов была описана с помощью микроскопии и путем засева на мясо-пептонный агар. Вначале целлюлозолитическую активность микроорганизмов тестировали в среде, содержащей аналитическую целлюлозу. Культивирование проводили в шейкере-инкубаторе в колбах Эрленмейра на 750 мл при температуре 30 °С и скорости перемешивания 200 об/мин в течение 6 дней. В результате консорциум разложил 49,2 % целлюлозы и показал лучшую целлюлозолитическую активность. Для дальнейшего эксперимента было проведено культивирование на среде, содержащей отходы твердой фракции послеспиртовой барды в качестве источника целлюлозолитических отходов. Содержание целлюлозы составило 20,78 %. При температуре 30 °С и скорости перемешивания 200 об/мин биодеградация целлюлозы составила 70,24 % в течение 6 дней с использованием консорциума целлюлозолитических микроорганизмов.

Ключевые слова: целлюлоза, биоконсервация, редуцирующие сахара, *Aspergillus awamori, Bacillus subtilis.*

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Determination of *Helicobacter pylori* by ELISA: single-center experience

Identifying the causes of helicobacteriosis, their timely diagnosis, and the choice of the correct treatment tactic will help improve the population health and increase life expectancy. This article is devoted to studying the presence of *Helicobacter pylori* antigens in the blood plasma of patients living in the Zhezkazgan city. The aim of the project is to determine the distribution of *Helicobacter pylori* by ELISA method. The result of our research shows that the presence of antibodies to *Helicobacter pylori* was found in 20 blood plasma samples from 45 analyzed, which is 44.45 % from overall samples number. The majority of positive results among Zhezkazgan city residents were detected in female samples (54.5 %), then in male (34.7 %) while in two previous studies the prevalence of positive results was in men or identical percentages in men and women. The most of positive results were detected in the residents at the age from 0 to 18 (50 %) and at the age who over 60 (66.6 %). The results identified that in Zhezkazgan city the Helicobacter pylori infection prevailed in the category of preschoolers and scholars and also among the old people, while the working population has the lower positive results (39.2 %).

Keywords: Helicobacter pylori, distribution, ELISA, antibody.

Introduction

Conducting a study on the presence of infectious disease pathogens such as *Helicobacter pylori* (*HP*) in Kazakhstan is becoming an increasingly relevant topic. *HP* pathogen is one of the most serious problems of gastroenterology due to the fact, that the prevalence of *HP* infection is progressively increasing, the disease is increasingly detected in people of young working age, as well as the fact that the microorganism is recognized as a first-order carcinogen. Consequently, the development of algorithms for early and accurate diagnosis of *HP* pathogens will improve the quality of treatment and follow-up of this category of patients. As well, more and more attention is being paid to the problem of re-infection, in connection with which it is necessary to clarify the timing of the control tests for *HP* to differentiate the re-infection and the failure of eradication therapy. *HP* is a helical gram-negative, microaerophilic bacterium that infects the areas of the stomach and duodenum [1–3]. For the last twenty five years, *HP* has been at the center of attention of scientists and gastroenterologists of the world. According to A.S. Bazhikova Kazakhstan belongs to countries with a high level of infection with *HP* — from 67.5 to 92 %, therefore, the solution of the issues of prevention and eradication of *HP* infection. It is an urgent medical and biological task for the country. For comparison, in Russia, the infection of the adult population ranges from 50 to 80 %, and in some regions it approaches 100 %. In Almaty, among adults with chronic gastritis, *HP* infection was 70 %, among adolescents — 60 %, *HP*-associated peptic ulcer disease in adults was found in 90 %, in adolescents — 98 %, *HP* etiology had 90 % of cases of duodenal ulcer disease intestines and 70 % of gastric ulcer. If in the southern region CagA-positive strains of *HP* were detected in 49.5 %, in the western region, Cag-A infected with *HP* were detected in 83 % of patients [4]. Unfortunately, there is no data on the frequency of infection with cytotoxic strains among ethnic populations

and indigenous people of Kazakhstan. The aim of the project is to determine the distribution of *HP* in Zhezkazgan city by ELISA method.

Research materials and methods

For detection of *Helicobacter pylori* by ELISA method human blood plasma of residents who applied to the "Zhezkazgan medical center" were used. Blood plasma samples were isolated by centrifuging 2300 rpm for 15 minutes. ELISA was carried out by using "Helicobacter pylori-CagA-antibodies-ELISA-BEST" reagent set (Vector-best, Russia). Optical density was measured on a spectrophotometer (BIO-RAD). The results of the analysis were checked through the analyzer and sent to the laboratory information system.

Research results and their discussion

In order to study the prevalence of Helicobacter pylori in the Zhezkazgan city, we determined the presence of antibodies to these pathogens in the blood plasma of residents who applied to the "Zhezkazgan Medical Center" within 2 months from March 4 to April 27, 2019.A total number of tested samples for *HP* were 45, from which 23 belong to men and 22 to women. The average age was 33.9±18 years old. 14 people of 45 tested were at the age from 0 to 19, 28 — at the age from 19 to 60 and 3 — at the age of over 60 years old (Table 1).

Table 1

Patients' characteristics and results of the analysis

№	Age	Sex	Helicobacter pylori	№	Age	Sex	Helicobacter pylori
1	67	F	+	24	57	M	–
2	44	M	+	25	40	F	–
3	12	M	+	26	65	M	+
4	29	F	+	27	12	M	–
5	4	F	+	28	51	F	–
6	46	F	+	29	31	F	–
7	44	F	+	30	54	M	–
8	20	F	–	31	20	M	–
9	32	M	+	32	15	F	+
10	49	F	+	33	47	M	–
11	27	M	–	34	14	F	–
12	18	F	–	35	27	M	–
13	49	M	–	36	16	F	+
14	16	F	–	37	14	M	+
15	47	F	–	38	54	M	–
16	40	F	+	39	55	M	–
17	15	M	–	40	70	F	–
18	13	F	+	41	12	M	–
19	25	M	–	42	16	F	–
20	37	M	+	43	59	F	+
21	34	M	+	44	51	M	–
22	21	F	–	45	45	M	+
23	11	M	+				

Helicobacter pylori was found in 20 plasma samples from 45 analyzed, which are 44,45 % from overall samples number. This was 23 cases per 100,000 people. Our result was similar with the analogical study in the southern region of Kazakhstan where positive results were detected in 49.5 % of tested samples, while in the western region of Kazakhstan positive results were detected in 83 % of samples [4]. In Atyrau city *HP* was detected in all patients (100 %) who applied to medical center № 1 [5].

Further studies suggested determining the prevalence of this pathogen among men and women.

The positive results on the presence of *HP* infection was determined in 8 men blood of 23 tested (34,7 %) and in12 women blood of 22 tested (54,5 %). According to our result the majority of positive results were detected in female samples.

Our result were different from the result received from the study conducted in the southern region of our country where the prevalence of Helicobacter pylori showed the similar percentages of positive result among

males and females for 2001 and the prevalence of positive result in middle-aged men (41.0 % of cases) than in women of the same age (24.5 %) for 2011 [6].

Further results suggested the determination of the prevalence of this pathogenin the different age categories. We divided all residents in three age categories: children, workers, and old people.It has been tested 14 people at the age from 0 to 18 years, 28 people at the age from 19 to 60 years and 3 people over 60 years old.The results are presented in figure.

Our study showed that 7 of all tested samples were with positive results in the residents at the age category from 0 to 18 which is 50 %, 11 positive results — at the age category from 19 to 60 which is 39.2 % and 2 positive results — over 60 years old which is 66.6 %.

The results received in our study were different from the result of the study conducted in the southern region of Kazakhstan where the prevalence of *HP* was found in the age group from 10 to 19 years (67 %) and in the age group from 49 to 59 years (93 %) [6].

Our study showed that 7 of all tested samples were with positive results in the residents at the age category from 0 to 18 which is 50 %, 11 positive results — at the age category from 19 to 60 which is 39.2 % and 2 positive results — over 60 years old which is 66.6 % (Fig. 1).

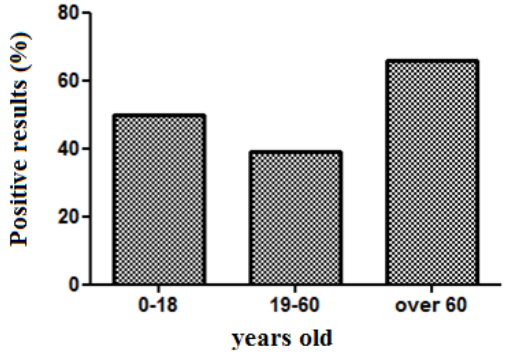

Figure 1. Positive results of Helicobacter pylori determination in different age categories

The results received in our study were different from the result of the study conducted in the southern region of Kazakhstan where the prevalence of *HP* was found in the age group from 10 to 19 years (67 %) and in the age group from 49 to 59 years (93 %) [6].

The results of our researches showed that in Zhezkazgan city the *HP* infection prevailed in the age category from 0 to 18 and among the old people, while the working population has the lower positive results.

The prevalence of this infection in children category is the big problem, because if it is left untreated, the bacteriumlives in the body indefinitely, which lead to the gastric and duodenal ulcers, gastritis, duodenitisor even stomach cancer in adult age. Incidence of Helicobacter pylori infections in children from 2 to 8 years in developing countries is 10 % per year and reaches almost 100 % of adult age [6].

In addition to age, an important factor in Helicobacter pylori is the socioeconomic position. Low socioeconomic status of the population causes the higher risk of infection.

Conclusion

So, the result of our research shows that the presence of antibodies to Helicobacter pylori was found in 20 blood plasma samples from 45 analyzed, which is 44.45 % from overall samples number. The data of our study show the similarity of total positive results with the southern region of our country.

For the detection of *Helicobacter pylori* were tested 23 men and 8 of them showed positive results (34.7 %) and 22 women and 12 of them showed positive results (54.5 %).The majority of positive results among Zhezkazgan city residents were detected in female samples, while in two previous studies the prevalence of positive results were in men or identical percentages in men and women.

Additionally, our study showed that most of positive results were detected in the residents at the age from 0 to 18 (50 %) and at the age who over 60 (66.6 %). The results identified that in Zhezkazgan city the Helicobacter pylori infection prevailed in the category of preschoolers and scholars and also among the old people, while the working population has the lower positive results.

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ИФА әдісімен *Helicobacter pylori* анықтау: бір орталықтың тәжірибесі

Хеликобактериоздың пайда болу себептерін анықтау, оларды уақтылы диагностикалау және емдеудің дұрыс тактикасын таңдау халықтың денсаулығын жақсартуға және өмір сүру ұзақтығын арттыруға көмектеседі. Мақала Жезқазған қаласында тұратын науқастардың қан плазмасында *Helicobacter pylori*-ге антиденелердің болуын зерттеуге арналған. Жобаның мақсаты ИФА әдісімен *Helicobacter pylori* таралуын анықтау болып табылады. Біздің зерттеулеріміздің нәтижелері көрсеткендей, *Helicobacter pylori* антиденелерінің болуы сарапталған 45 қан плазмасының 20 үлгісінде анықталған, бұл үлгілердің жалпы санының 44,45 %-ын құрайды. Жезқазған қаласының тұрғындары арасында оң нәтижелердің көпшілігі ерлер тобына қарағанда (34,7 %) әйелдердің сынамаларында (54,5 %) анықталды, ал алдыңғы екі зерттеулерде оң нәтижелердің басым бөлігі ер адамдарға немесе әйелдер мен еркектерде бірдей болған. Жоғары оң нәтижелер 0–18 жас аралығындағы (50 %) және 60 жастан асқан (66,6 %) тұрғындарда анықталған. Нәтижесінде Жезқазған қаласында *Helicobacter pylori* жұқпасы мектепке дейінгі балалар мен оқушылар, сондай-ақ қарт адамдар арасында басым болды, ал еңбекке қабілетті халықтың оң нәтижелері (39,2 %) төмен болды.

Кілт сөздер: Helicobacter pylori, таралуы, ИФА, антидене.

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Определение *Helicobacter pylori* методом ИФА: опыт одного центра

Выявление причин возникновения хеликобактериоза, своевременная их диагностика и выбор правильной тактики лечения способствуют улучшению здоровья населения и увеличению продолжительности жизни. Данная статья посвящена изучению наличия антител к *Helicobacter pylori* в плазме крови больных, проживающих в г. Жезказгане. Целью проекта является определение распространения *Helicobacter pylori* методом ИФА. Результаты наших исследований показали, что наличие антител к *Helicobacter pylori* было обнаружено в 20 образцах плазмы крови из 45 проанализированных, что составляет 44,45 % от общего количества образцов. Большинство положительных результатов среди жителей г. Жезказгана было выявлено в женских выборках (54,5 %), чем в мужских (34,7 %), в то время, как в двух предыдущих исследованиях, заметно преобладание положительных результатов у мужчин или наблюдались одинаковые проценты у мужчин и женщин. Наибольшие положительные результаты были выявлены у жителей в возрасте от 0 до 18 лет (50 %) и в возрасте старше 60 лет (66,6 %). Кроме того, в г. Жезказгане инфекция *Helicobacter pylori* была широко распространена в категории дошкольников и школьников, а также среди пожилых людей, в то время как у трудоспособного населения положительные результаты были ниже (39,2 %).

Ключевые слова: Helicobacter pylori, распространение, ИФА, антитела.

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Оқшауланған гепатоциттердің жасушалық гомеостазын сақтауда аутофагияның ролі

Мақалада оқшауланған гепатоциттердің ультрақұрылымдық ұйымы зерттелген және олардың өсу динамикасындағы жасушаішілік өзгерістердің сипаты анықталған. Нативтік қасиеттері сақталған гепатоциттерді өсіру әдісін әзірлеу, әртүрлі сыртқы факторлардың, химиялық қосылыстардың және жаңа дәрілік препараттардың гепатотоксикалық әсерлерін зерттеу үшін және бауырдың тұқым қуалайтын немесе терминалды ауруларын емдеуде жасушалық трансплантацияны жүргізуге мүмкіндік беретін қажеттілігімен анықталды. Бастапқы гепатоциттерді өсіру үлгілері бауыр метаболизмін, секрециясын және регенерациясын зерттеу үшін қолданылады. Бастапқы гепатоциттерді өсіру стандартты қоректік ортада жүргізілген, ал өсу динамикасы ағынды цитофлуориметрия, жарық және электронды микроскоптар арқылы зерттелген. Стандартты қоректік ортада өсірілген бастапқы гепатоциттерді зерттеу нәтижесінде, гепатоциттердегі базальды аутофагияның жоғарылауы гликофагия мен митофагия түрінде көрініс берді. Аутофагия стандартты өсірілген жағдайда оқшауланған гепатоциттердің жасушалық гомеостазын сақтауға мүмкіндік береді.

Кілт сөздер: оқшауланған гепатоциттер, базальды аутофагия, жасушалық цикл.

Кіріспе

Соңғы уақытта бастапқы гепатоциттер жасушаларын өсіру бауырдың тұқым қуалайтын немесе терминалдық ауруларын емдеуде трансплантация жүргізу қажеттілігі туындауына байланысты қарастырылуда [1]. Бауырдың ерекшелігі - патологиялық факторлар жойғаннан кейін, бауырдың қалыпты құрылымының қалпына келуі [2]. Бауырда көптеген функцияларды — паренхиматозды жасушалар немесе гепатоциттер атқарады [3]. Бауырдың паренхиматозды жасушасы — гепатоцит күрделі, энергетикалық қарқынды, поляризацияланған эпителиальды жасушалар болып табылады [1]. Гепатоциттер регенерация кезінде ағзаның тіршілігін қамтамасыз ететін бауырдың маңызды функцияларын сақтай отырып, 1000-нан астам гендерді экспрессиялайды [4].

Бауыр метаболизм мен залалсыздандырудың орталық органы болып табылатындықтан, оқшауланған гепатоциттер дәрілік препараттардың фармакологиялық және токсикологиялық реакцияларын анықтау үшін үлгі ретінде пайдаланылады [5]. Сонымен қатар, бастапқы гепатоциттерді өсірудің оңтайлы әдісі мен тиімділігін анықтау мәселелері сақталуда [6].

Жасушаішілік немесе жасушадан тыс микроортаның өзгеруі жағдайында жасушалардың гомеостазын сақтау механизмі аутофагия болып табылатыны белгілі [7]. Аутофагиялық механизм жасушадан тыс микроортаның метаболикалық өзгерістеріне өте сезімтал және стрестік жағдайларды жеңу үшін бейімделген аутофагиялық жауап маңызды болады [8]. Аутофагия және апоптоз — жасушаішілік екі қарама-қарсы үрдіс. Аутофагия — жасушаның тіршілігін сақтап қалу тәсілі [9], ал апоптоз — жасушаның жойылу түрінің бірі [10]. Аутофагия жасушалық гомеостазды сақтау және энергия өндіруге арналған субстраттарды қамтамасыз ететін лизосомалардағы дұрыс оралмаған ақуыздар мен зақымдалған цитоплазмалық компоненттердің тозу жолын білдіреді [11].

Зерттеу жұмысының мақсаты — оқшауланған гепатоциттердің цитоплазмасындағы базальды аутофагияны, оларды өсіру динамикасында зерттеу.

Зерттеу материалдары және әдістері

Гепатоциттерді бөліп алу және өсіру. Зерттеу бастапқы өсірілген гепатоциттерде жүргізілді. Салмағы 180–200 г. Вистар саласындағы аталық егеуқұйрықтардың гепатоциттері 0,03 % коллагеназа

ерітіндісін («ICN Biomedicals, Inc», АҚШ) пайдалана отырып, рециркуляторлы ферментативті перфузия әдісін қолданып, дифференциалды центрифугалау арқылы паренхимиялық емес жасушалардан бөлініп алынды. Жасушалардың тіршілік ұзақтығы трипанды көкті («Serva», Германия) жасушалар құрамынан шығару әдісімен бағаланды. Экспериментке тіршілік ұзақтығы 90 %-дан кем емес жасушалар алынды. Алынған жасушаларды концентрациясы $10 \cdot 10^4$ жасуша/ойық коллагенмен жабылған 6-ойығы бар планшеттерге (Corning) отырғызылды. Гепатоциттер келесі қоректік ортада RPMI-1640 (Gibco, АҚШ), pH 7,4, құрамында 10 % сиырдың эмбрионалды сарысуы (Gibco, АҚШ), 100 бірл./мл пенициллин, 50 мкг/мл гентамицин стандартты жағдайларда (5 % CO_2, 37 °С температурада және 95 % ылғалдылықта) өсірілді.

Өсірілетін гепатоциттердің жасушалық циклын бағалау. Өсірілген гепатоциттердің жасушалық циклын талдау үшін Пропидий йодидінің (PI) интеркалибрлеуші флуоресцентті бояғыш ДНҚ қолдану арқылы, ағынды цитофлуориметрия әдісі қолданылды. Жасушалар 1, 24 және 48 сағат бойы өсірілді. Жасушаларды пластиктен алу үшін TrypLE реагентті (Gibco, АҚШ) пайдаланылды, центрифугалаумен жасушалар тұнып, фосфатты-тұзды буфермен (PBS) жуылды және мұздатылған 70 % этанолмен бекітілді. ДНҚ экстракциясы үшін буфермен инкубациядан кейін жасушалар қайтадан центрифугаланып және PBS-пен жуылды. Пропидий йодидімен боялған жасушалар CytoFlexS (Beckman Coulter, АҚШ) ағынды цитофлуориметрмен талданды.

Трансмиссиялық электронды микроскоп. Гепатоциттердің ультрақұрылымдық ұйымдастырылуын зерттеу үшін Хенкс ортасында дайындалған параформальдегидтің 4 % ерітіндісінде жасушаларының белгілі мөлшері фиксацияланды, одан кейін 1 сағат ішінде фосфатты буферде (pH=7,4) 1 % OsO_4 ерітіндісінде тағы да фиксация жүргізілді, дегидратацияны этил спиртінің ұлғаю концентрациясында жүргізіп, эпонмен (Serva, Германия) қапталды. Қалыңдығы 1 мкм болатын жартылай жіңішке кесінділер Leica EM UC7 (Leica Microsystems, Германия) ультрамикротомында дайындалды, толуидинді көкпен боялып, сәулелі микроскоп «LEICA DME» (Leica Microsystems, Германия) арқылы зерттелді. Қалыңдығы 70–100 нм болатын ультражіңішке кесінділерді сулы ерітіндіде қаныққан уранилацетат және қорғасын цитратымен контрастылығын келтіріп, электронды микроскоппен JEM 1010 (JEOL, Жапония) зерттелді.

Морфометрия және статистикалық мәліметтерді өңдеу. Морфометриялық талдау ImageJ (Wayne Rasband, АҚШ) компьютерлік бағдарламаның көмегімен жүргізілді. Гепатоциттердің ядролары мен цитоплазмасының диаметрі, ядролар мен цитоплазманың көлемі және ядролық-цитоплазмалық арақатынасы анықталды. Жасушаішілік органеллалардың концентрациясын 500 нүктеден тұратын жабық тест жүйесін пайдалана отырып, ×30000 ұлғайған кезде бағаланды. Орташа мән (M) және стандартты ауытқу (SD) Microsoft Excel (Microsoft, АҚШ) бағдарламалық жасақтамасын пайдалану арқылы есептелді. Зерттелетін параметрлер арасындағы айырмашылықтардың анықтығы 95 % ($P<0,05$) сенімділік деңгейінде U-критерия Манна-Уитни көрсеткішін қолдану арқылы Statistica 6.0 (StatSoft, АҚШ) бағдарламалық қамтамасыз ету көмегімен анықталды.

Зерттеу нәтижелері және оны талдау

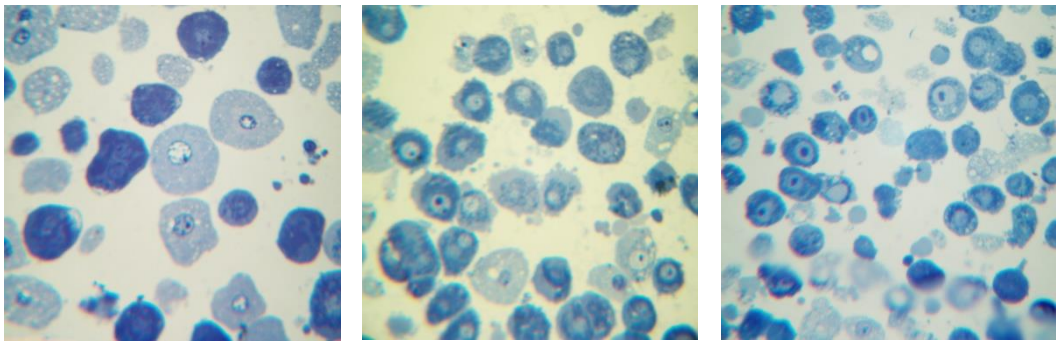
Оқшауланған гепатоциттерді өсіру кезінде 48 сағат ішінде жасушалардың абсолюттік көлемінің біртіндеп төмендегені байқалды. Өсірілген гепатоциттер жасушасының көлемі 24 сағаттан кейін 57 %-ға кеміді, ал 48 сағаттан кейін 76 %-ға кеміді, 1 сағат өткендегі жасушалар көлемімен салыстырғанда. Бұл ретте гепатоциттер ядроларының абсолюттік көлемі нақты өзгерген жоқ. Ядролық-цитоплазмалық арақатынас 24 сағаттан кейін 2 есе, 48 сағаттан кейін 4 есе артты (1 А-Б сурет). Демек, өсіру процесінде гепатоциттер көлемінің төмендеуі, жасушалардың цитоплазмасының көлемдік үлесінің төмендеуі есебінен жүрді.

Жасушалық циклды бағалау стандартты қоректік ортада өсіру кезінде 24 сағаттан кейін G0/G1-де гепатоциттердің тоқтауын көрсетті. 48 сағат ішінде зерттеу апоптоз сатысындағы жасушалар пайызының көтерілмегенін, гепатоциттер өздерінің тіршілік ұзақтығын сақтап қалғанын айқындады (2-сурет).

Гепатоциттердің ультрақұрылымдық ұйымдасуын зерттеуде 24 сағаттық өсіруден кейін гепатоциттердің цитоплазмасында табақша тәрізді гликогендер мен цитоплазма фрагменттерінде аутофагосомалар (3А сурет) және ішінара тозған материалмен аутолизосом анықталды (3Ә сурет).

Егер 24 сағаттық өсіруден кейін аутофагосом құрамында гликогеннің түйіршіктері байқалса, онда гепатоциттердің ультрақұрылымдарын 48 сағаттан кейін зерттеу кезінде цитоплазма және митохондрияның фрагменттері бар аутофагосомалар анықталды (3Б, В сурет). Митохондриялар түйіршікті эндоплазмалық ретикулум цистерналарымен жиі қоршалған (3Г сурет). Сонымен қатар, митохондрия-

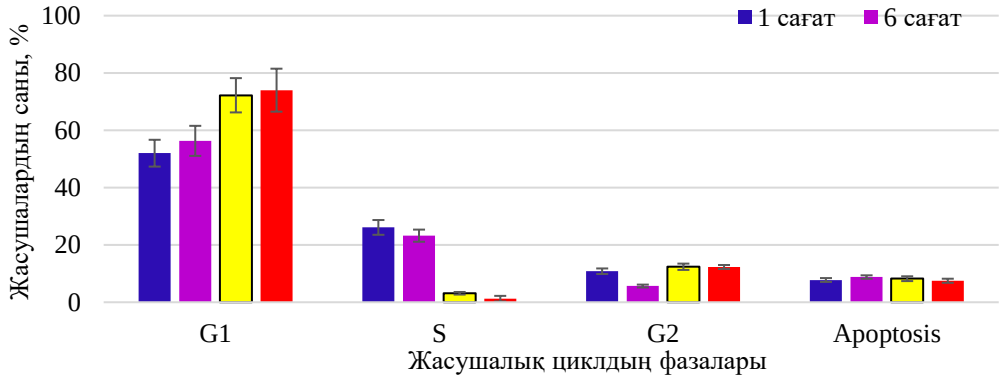
лардағы кристалар құрылымының бұзылу және ыдырап-бұзылудың әртүрлі сатысындағы мембраналық құрылымдардың қосылуымен аутолизосомалардың санының жоғарылауы байқалды (3Д сурет).



А *Ә* *Б*

А, Ә, Б — 1, 24 және 48 сағаттан кейін, гепатоциттерді сәйкесінше өсіру. Толуидинді көкпен боялған. Ұлғайтылған ×400

1-сурет. Оқшауланған гепатоциттерді өсіру динамикасының морфологиясы



2-сурет. Гепатоциттерді стандартты қоректік ортада өсіру кезінде жасуша циклінің фазалары бойынша таралуы

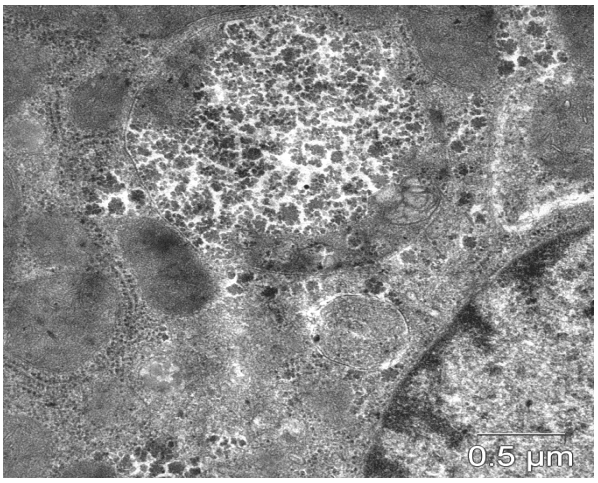
Гепатоциттердің ультрақұрылымдық ұйымдасуына 48 сағат ішінде морфометриялық зерттеу жүргізу, өсірілген гепатоциттерде гликогеннің және митохондрияның көлемдік тығыздығы 84 % және 27 % ($p<0,05$) сәйкес төмендегенін көрсетті (1-кесте). Бұл ретте, аутофагосом және аутолизосомның көлемдік тығыздығы 50 % және 7 есеге ($p<0,05$), сәйкес артты (1-кесте).

1 - к е с т е

Гепатоциттерді өсіру динамикасындағы морфометрияның нәтижелері (M±SD)

Параметрлері	1сағат	24 сағат	48 сағат
Гепатоциттер, V (мкм^3)	8089,77±3465,86	7322,782±3682,01*	4154,77±1904,82*
Гепатоциттер ядросы, V (мкм^3)	423,04±137,21	427,73±127,91	514,83±124,8
ЯЦИ	0,052±0,0215	0,074±0,0515	0,179±0,0315*
Митохондрия, Vv (%)	14,2±2,72	14,7±1,78	10,4±2,38*
ЭПР, Vv (%)	4,41±0,43	3,92±1,04	2,02±0,56
Аутофагосомдар, Vv (%)	5,87±1,45	21,2±5,13*	8,78±3,56*#
Аутолизосомдар, Vv (%)	0,94±1,22	2,65±2,51*	6,88±2,21*#
Гликоген, Vv (%)	5,41±1,14	1,42±1,08*	0,86±0,84*

Ескертпе. Vv — құрылымдардың көлемді тығыздығы; ЭПР — эндоплазматикалық ретикулум; ЯЦИ — ядролық-цитоплазмалық индекс (VV ядро/VV цитоплазмалар). 1, 24, 48 сағат — гепатоциттерді өсіру уақыты. * — 1 сағ. өсіру арқылы сәйкес шамалардан айырмашылығы, # — 24 сағ. өсіру арқылы сәйкес шамалардан айырмашылығы ($p\leq0,05$).



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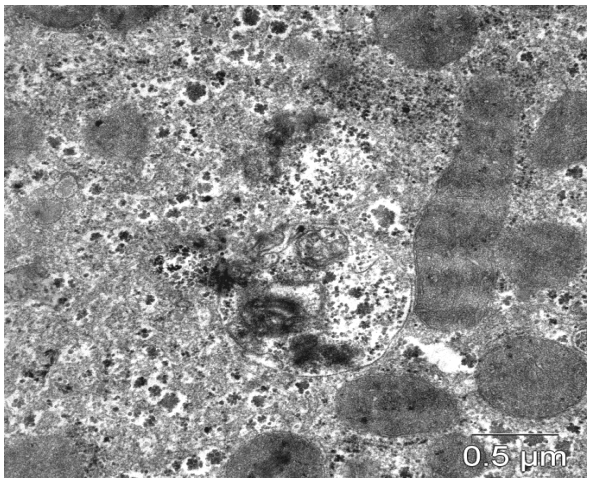

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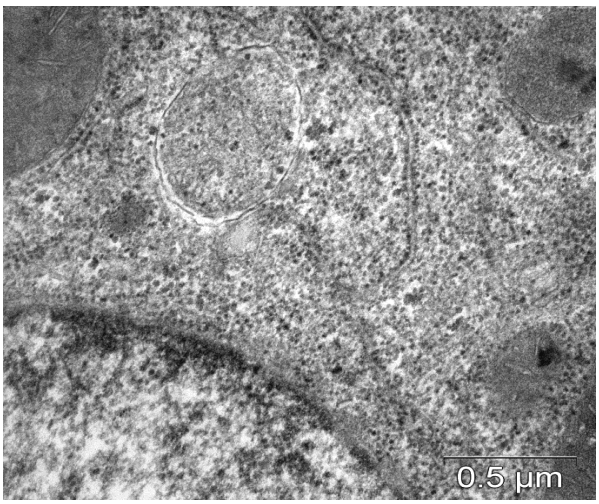

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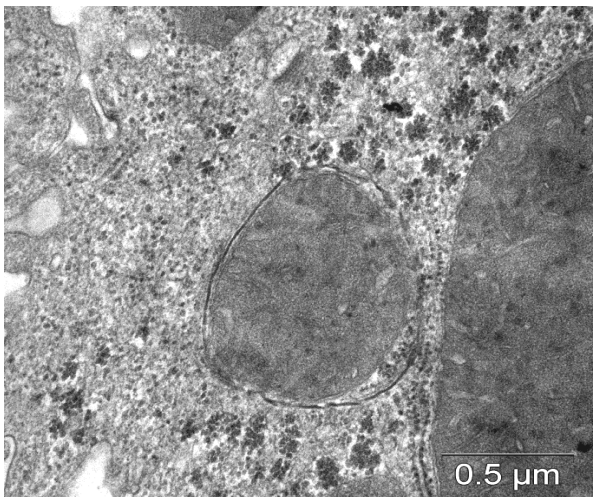
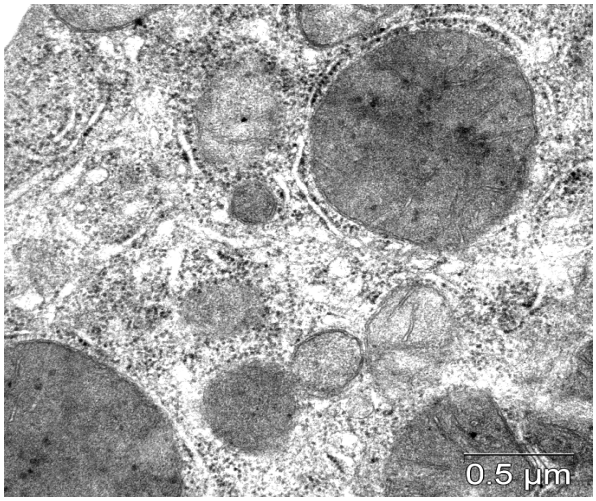


В



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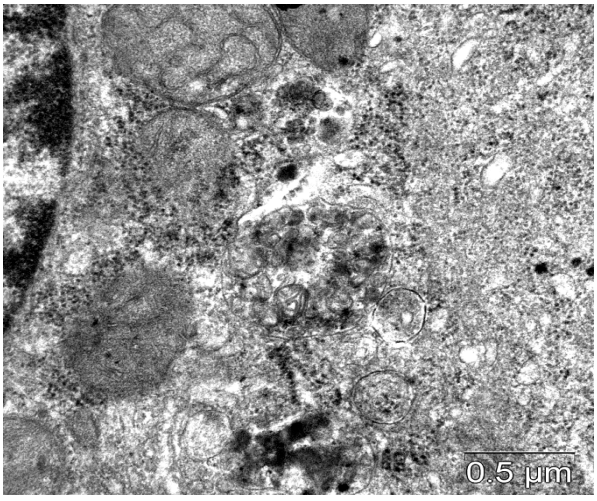

Д

А — 24 сағаттық өсіруден кейін аутофагосомадағы табақша тәрізді гликогендер; *Ә* — 24 сағаттық өсіруден кейін ішінара тозған материалдары бар аутолизосома; *Б* — 48 сағаттық өсіруден кейін цитоплазма фрагменті бар аутофагосома; *В* — 48 сағаттық өсіруден кейін митохондриясы бар аутофагосома; *Г* — 48 сағаттық өсіруден кейін цитоплазмада гликогеннің болмауы және митохондрия айналасында эндоплазмалық ретикулум сақиналарының пайда болуы; *Д* — 48 сағаттық өсіруден кейінгі бұзылудың әртүрлі сатысындағы мембраналық құрылымдарды қосумен аутолизосомның жоғары саны

3-сурет. Өсіру процесі кезіндегі гепатоциттердің ультрақұрылымдық ұйымдасуы

Алынған мәліметтерді талқылау

Аутофагия макромолекулалық ақуыз агрегаттарын, қосалқы қоректік заттардың жасушалық органеллаларын (гликоген мен липидтер) жоюға және тозуына бағытталған, жасушалық гомеостазды ұстап тұруға және стресс жағдайында белсендіретін, катаболикалық бағдарлама болып табылады. Аутофагия нәтижесінде лизосомаларда түзілетін метаболиттер макромолекулаларды синтездеу үшін энергия көздері немесе құрылыс блоктары ретінде қайта пайдаланылады [12].

Гепатоциттер энергетикалық ресурс — гликогеннің түзілуі мен сақталуында маңызды, шешуші рөл атқаратыны белгілі [13]. Әдебиет деректері аутофагия мен көмірсулар алмасуы арасындағы өзара әрекеттесуді және аутофагия мен жасушалық энергетикалық баланс арасындағы динамикалық кері байланыстың болуын көрсетеді [12]. Гликоген аутофагосомалармен танылуы және сіңірілуі мүмкін, содан кейін ыдырау үшін лизосомаға беріледі. Бұл процесс «гликофагия» деп аталады [14]. Біздің зерттеуде гепатоциттерді өсіру кезінде 24 сағаттан кейін гликогені бар аутофагосомалар басым болды, ал 48 сағаттан кейін митохондриясы бар аутофагосомалар пайда болды. Бұл кезеңде жасушалық циклдің S-фазасындағы жасушалардың саны ең аз болса, G0/G1 сатысындағы жасушалардың жоғары пайызының болуы анықталды. Аминқышқылдарының жетіспеушілігі жағдайында ақуыз синтезі мен митоз тоқтайтыны белгілі, ал аутофагиялық сигналдық жол тіршілікке маңызды ақуыздарды синтездеу үшін аминқышқыл пулының қолжетімділігін қамтамасыз ету мақсатында ақуыздарды ыдырату жолымен аминқышқылдарын босату үшін белсендіріледі [14].

Гепатоциттердің жасушалық культураларын медицинада пайдалануды шектейтін мәселелердің бірі өсіру кезінде жасушалардың митоздық белсенділігінің жоғалуы болып табылады [15]. Жасушалық циклді тоқтату біздің зерттеуде де көрсетілген. Сонымен қатар, өсірудің 48 сағатында гепатоциттердің базальды аутофагия деңгейі өсті және апоптоз жағдайындағы жасушалар саны ұлғайған жоқ. Бұл жағдайда аутофагия, гепатоциттердің дифференциялану деңгейін қолдап, жасушалық гомеостазды сақтаудың тиімді тәсілі болып табылды [16].

Қорытынды

Біздің алған мәліметтеріміз оқшауланған гепатоциттерді теңдестірілген қоректік ортада өсіру кезінде жасушалардың цитоплазмасында базальды аутофагияның өсуін көрсетеді. Тәжірибеден соң 24 сағаттан кейін гликофагия дамыды, өйткені аутофагосомаларда негізінен жасуша үшін энергия көзі болып табылатын материал — гликоген түйіршіктері болды. Гликофагияға қосымша, 48 сағаттан кейін жасушалардың амин қышқылдарына деген қажеттілігіне және ақуыз кешендерінің ыдырауына байланысты митофагия байқалды. Алынған мәліметтер гепатоциттердің бастапқы культурасының тіршілік етуіне аутофагияның қосқан үлесін көрсетеді және оларды өсіру жағдайларының жеткіліктілігінің көрсеткіші ретінде пайдалануға болады.

Сонымен, ағынды цитофлуориметрия, жарық және электрондық микроскопия әдістерімен стандартты қоректік ортада бастапқы гепатоциттердің өсіру динамикасы сипатталды. 24 сағаттан кейін жасушалық циклдің G0/G1 сатысында өсірілген гепатоциттердің тоқтауы және апоптоз сатысында сол жасушалардың пайызы көбеймей, олардың тіршілік ұзақтығы сақталғаны анықталды. Жасушалардың цитоплазмасында базальды аутофагияның артуы — гликофагия мен митофагияның басым болуы, гепатоциттерді өсіру процесінде жасушалық гомеостазды сақтау механизмі болып табылады.

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Роль аутофагии в сохранении клеточного гомеостаза изолированных гепатоцитов

В статье исследована ультраструктурная организация изолированных гепатоцитов и определен характер их внутриклеточных изменений в динамике их культивирования. Разработка методологии культивирования гепатоцитов с сохранившимися нативными свойствами позволяет изучить гепатотоксические эффекты различных внешних факторов, химических соединений и новых лекарственных препаратов, что необходимо для клеточной трансплантации при лечении наследственных или терминальных заболеваний печени. Модели первичной культуры гепатоцитов используются для изучения метаболизма, секреции и регенерации печени. Первичная культура гепатоцитов была исследована в динамике культивирования в стандартной питательной среде с помощью метода проточной цитофлуориметрии, световых и электронных микроскопов. Аутофагия изолированных гепатоцитов при стандартных условиях культивирования способствует поддержанию клеточного гомеостаза.

Ключевые слова: изолированные гепатоциты, базальная аутофагия, клеточный цикл.

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The role of autophagy in maintaining cellular homeostasis of isolated hepatocytes

The article investigates the ultrastructural organization of isolated hepatocytes and determines the nature of their intracellular changes in the dynamics of their cultivation. The development of a methodology for the cultivation of hepatocytes with preserved native properties allows us to study the hepatotoxic effects of various external factors, chemical compounds and new drugs, which is necessary for cell transplantation in the treatment of hereditary or terminal liver diseases. Primary hepatocyte culture models are used to study liver metabolism, secretion, and regeneration. The primary culture of hepatocytes was studied in the dynamics of cultivation in a standard nutrient medium using flow cytofluorometry, light and electron microscopes. Autophagy in isolated hepatocytes under standard culture conditions contributes to the maintenance of cellular homeostasis.

Keywords: isolated hepatocytes, basal autophagy, cell cycle.

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On the chemical mechanisms of interaction of diabetogenic toxic substances with zinc in the pancreas and methods for its prevention

Article presents data on chemical mechanisms of binding of Zinc in pancreas of animals and human by diabetogenic chelate active chemicals (DZC) that result destruction and death of pancreatic B-cells within a few minutes and of developing of diabetes mellitus. Authors presented and described a few mechanisms for prevention of binding of Zinc in pancreas that result prevention developing of experimental diabetes in 95–100 % of animals. The authors analyze and substantiate in details on the basis of own investigations (1964–2018), the chemical mechanisms of zinc blocking in cells, which prevents the possibility of their destruction caused by diabetogenic zinc-binding substances, the possibilities of human contact with which have significantly increased over the past decades. According to the results of our own studies and literature data, the chemical mechanisms of the preventive action of derivatives of Dithiocarbamic acid, amino acids — reduced glutathione, cysteine and histidine, as well as the possibility of chemical neutralization of the blood of the DCS before they reach the pancreas, were investigated.

Keywords: zinc, B-cells, Diabetogenic zinc binding chemicals, Glutathione, Diphenylthiocarbazone (Dithizon).

Abbreviationes: DZC — Diabetogenic zinc binding chemicals; DZ — Diphenylthiocarbazone (Dithizon); GRF — Glutathione restored form; GOF — Glutathione oxidised form; NaDDCA — Na salt of Diethyldithiocarbamic acid; 8TSQ — 8-para(toluenesulphonylaminoquinolin).

Introductrion

More than 80 years ago Scott and Fischer were separated insulin from the native pancreas as Insulin-Zn complex and supposed that the presence of Zn-ions determined physiological activity of insulin [1, 2]. Interest for this problem is increased after reporting that in pancreas of diabetic patients total amount of Zn is not more than 50 % in compared with non diabetic men. They found 0.07 mg of Zn per 1g of pancreas tissue of diabetic patients comparatively with 0.14 mg per 1g pancreas of healthy persons. Analogical result was obtained by Eisenbrandt and coll. [3]. A large amount of Zn^{+2}-ions was found in human pancreas of healthy men. Okamoto K. discovered in pancreatic B-cells a large amount of Zn^{+2} [4]. It is supposed today the important role of Zn-ions in processes of storage of insulin in B-cells [5, 6]. There are proportional dependence between content of Zn-ions in B-cells and in cytoplasm. Decreasing of content of deposited insulin accompanied by decreasing of amount of Zn-ions in B-cells. It is known that Zn-ions take part in processes of synthesis as in cristallization of insulin. It was showed that pancreas of mammals-animals, human, birds and in earth-water animals contained a large amount of Zn-ions.

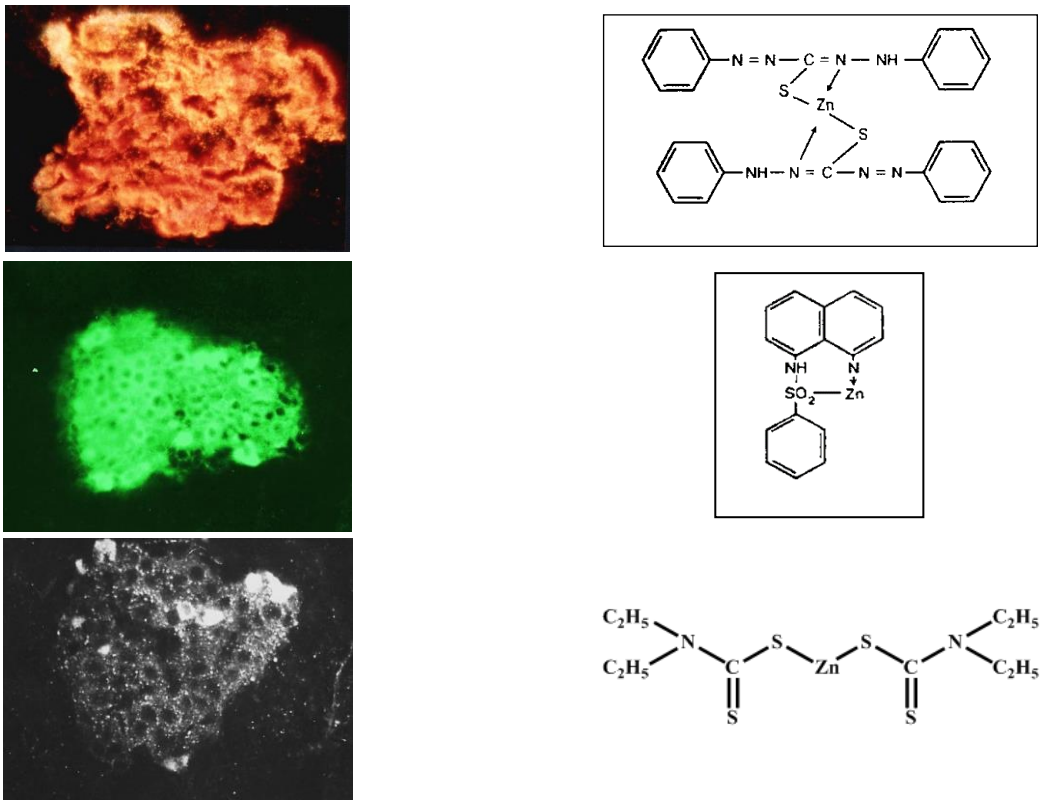
Today, more than 20 chemicals are known that can selectively damage B cells in the body, which leads to their rapid death. Of these, 18 substances belong to the group of diabetic zinc-binding substances that form in complex B cells intracellular salts (chelates) with zinc contained in B cells, which leads to their rapid death. The authors investigated the chemical mechanisms of the formation of zinc-chelator complexes [7]. It was shown which parts of the DCS molecules and through which atoms they form chelates with zinc. Based on the studies, it was shown that the preventive effect of zinc blocking by non-diabetogenic substances is realized through interaction with the sulfur and nitrogen atoms of the DZC, that is, through the same atoms with which the DCS form complexes toxic to cells. Regarding the preventive action of the amino acids glutathione and cysteine, it was shown that their preventive effect is due to the blocking of the zinc atom through the sulfur atoms of SH radicals, which prevents the interaction of zinc with DZC. The Zn-ions in cytoplasm of B-cells have the coordinate number 4 and 6 and interacted with chemicals which formed with Zn-ions chelat salts in

which atom of Zn^{+2} is fixed between a few other atoms [8]. The affinity of Zn-ions to formation of chelates is evidently more high comparatively with other metals of main group.

Diabetogenic activity of Zinc-binding chelators Dithizon and derivatives of 8-oxyquinoline

From the more than 20 chemicals that cause selective destruction of B-cells, 18 are represented by derivatives of 8-hydroxyquinoline and Dithizon (Diphenylthiocarbazone) (Fig.1).

Dithizon (diphenylthiocarbazon) is one of most active chelators [4, 9]. Dithizon formed various modifications of red colour chelates with 18 metals. It possesses a marked high affinity to Zn-ions and formed very rapidly past injection chelate 2:1 that accompanied by destruction and death of B-cells within 15–30 min. and developing of 1st type of diabetes 24–48 h later. It was showed that first changes in cytoplasm of B-cells appeared 5 min past injection of DZ as small zones of destruction of cytoplasm. More detail analysis using of transmission electron microscopy showed that process of destruction of B-cells started by destruction of B-granules.



From above: 1) red granules; chelat complex Zinc-Dithizon in B-cells and disposition of atom of Zinc in complex; result destruction and death of B-cells; 2) green fluorescence of chelat complex Zinc-8PTSQ in B-cells and disposition of atom of Zinc in complex; result destruction and death of B-cells; 3) white granules: chelat complex Zinc-NaDDCA; protect B-cells of destruction

Figure 1. Various chelat complexes Zinc-chelator

For the first, the 2–3 B-granules are destructed with forming of small zones of destruction of cytoplasm of B-cells [10], not more than 3–5 % of total surface of section of B-cells. 15 min later the sizes of these zones rapidly increased until 30–40 % of surface of B-cells and 1–2 h past injection almost all cell's matrix, 80–90 % of section's surface, is destroyed completely. It is showed that these changes are not visible on light microscopy but very well discovered by transmission electron microscopy. Destructive histological changes developed a few days later — are secondary changes as result of not visible destroying of B-cells within first few minutes after forming of chelate complex in cytoplasm of B-cells. Thus, it was concluded that destruction of B-cells past injection of diabetogenic doses of Dithizon and of 8TSQ is determined by action of complex Zn-DZ and Zn-8TSQ on structure, for the first, on B-granules of B-cells, where is concentrated zinc as deposited form "zinc-insulin complex" within first 15–30 min. past forming of complex in cytoplasm of B-cells (Fig. 1).

Diabetogenic derivatives of 8-oxyquinoline

A. Albert in 1947 reported that 8-oxyquinoline which usually belong to not toxic substances, is very toxic for cells in the presence of metals and for the first time — of Zn-ions. It was showed that this fact determined by ability of 8-oxyquinoline to form with metals the chelate metal-complexes which are toxic for B-cells [11, 12] as complexes formed in B-cells by other chelate active substance as Dithizon. Studying of toxicity of 8-oxyquinoline for B-cells K. Okamoto [9] reported that injection of it to animals accompanied by developing of experimental diabetes. Later it was showed that injection of 18 derivatives of 8-oxyquinoline and of 8-oxyquinaldin accompanied by rapid developing of heavy diabetes in animals. It was noted that all these chemicals have in position 8 of quinoline ring OH-group or any other radical contained atom of S or atom of O. Six isomers of 8-oxyquinoline not contained in position 8 of the active group are not able to form chelate complexes with Zn-ions and not induced experimental diabetes. Experimental diabetes is induced by derivatives as 8-para(toluenesulphonylamino)quinoline /8PTSQ/, 8-para(benzenesulphonylamino)quinoline /8PBSQ/, 8-para(methansulphonylamino)quinoline /8PMSQ/, 5-para(acetaminophenylazo)-8-oxyquinoline /5A8OX/, 8-hydroxyquinaldin, 5-amino-8-hydroxyquinoline and others (Fig. 2, 3). It was demonstrated [9] that injection of these derivatives result selective necrosis of B-cells and developing of diabetes. Injection of these chemicals in doses of 30–100 mg/kg accompanied by developing within a few days of heavy diabetes with marked degenerative changes in islets.

On the chemical mechanisms of binding of Zinc-ions by derivatives of 8-oxyquinoline

It is known that most stable complexes are formed when atom of Zn is fixed between 2 atom of N, S and O of molecule of chelator. Later it was reported that only derivatives of 8-oxyquinoline contained in position 8 of quinoline ring of the hydroxyl or other radical contained atoms of S, N or O possess diabetogenic properties. Atom of Zn is fixed between atoms of O in position 8 and of N in position 1 or between two atoms of O in positions 2 and 8 (xanthurenic acid) (Fig. 3).

It was reported, what is more, that extraction of these radicals from position 8 accompanied by complete disappearing of diabetogenic properties of chelators [9]. Formation of chelats by atoms of O and N of chelator result usually forming of pentagonal or hexagonal rings [8, 9] (Fig.3). Pentagonal rings are more stable. The most stable are quadrangular complexes with atom of S. Electrons of indivisible pair are displaced from donor atom of N in position 1 to Zn atom.

On the base of data obtained by A. Albert, it was supposed that toxic effect of 8-oxyquinoline is determined by its ability to bind and eliminate ions of metal from B-cells. But later this hypothesis was not confirmed: it was showed that long time prolonged elimination of Zn-ions from B-cells result any effect on the state of histostructure and function of B-cells [10].

Finally, S. Rubbo and A. Albert established that toxic effect of 8-oxyquinoline determined by its ability to form in cells toxic complexes with metals [11] that many times was confirmed later. It was showed that presence of chelate a short time in cytoplasm of B-cells accompanied by alteration of cells. In experiences with using derivatives of 8-oxyquinoline — a various isomers of the azaoxyquinoline (azaoxyn) — it was demonstrated dependence: most toxic are isomers formed chelats 1:1 with metal have logarithm of constant of stability as 7.6 and more high, until 9.4. Meanwhile toxicity of chelats of other isomers of azaoxyn with constant of stability 5.8–6.7 were clearly more less [12]. It was showed that very toxic chelats of derivatives of 8-oxyquinoline with Zn-ions have a more high logarithm of constant of stability as 8.5. Weitzel G. and coll. showed that complex 1:1 contained 1 molecule of 8-oxyquinoline and 1 atom of ion of Zn is most toxic for cells [13].

Stability of formed complexes 2:1, as complex Dithizon-Zinc, is depended not only of affinity of chelator to metal but in added — by 2 properties of chelator and metal: 1) presence of additional radicals in para-positions molecule of chelator, especially — in zones which contacted with part of molecule, reacted with ions of metal conduce to forming of the steric effect; as result, two molecules of chelator are not able to approach for to put atom of metal in stable ring; 2) size of diameter of atom; in case if atom of metal have a small diameter, ring may be not formed; atom of Zn have radius as 0.74 nm between Berillium (0.31 nm) and Rubidium (1.49 nm). A high stability of complex Zn-Dithizon is determined by stretch form of molecule of Dithizon and by location of 2 phenol rings on the 2 ends of molecule. That is why atom of N and S are easy approach to atom of Zn. More over, atom of Zn is fixed between atoms of N and S. Meanwhile it is known that affinity of Zn to N and S is more high comparatively with affinity of Zn to O. In added, complex is formed by two molecule of Dithizon each of two have a great number of double couplings **(**Fig. 3).

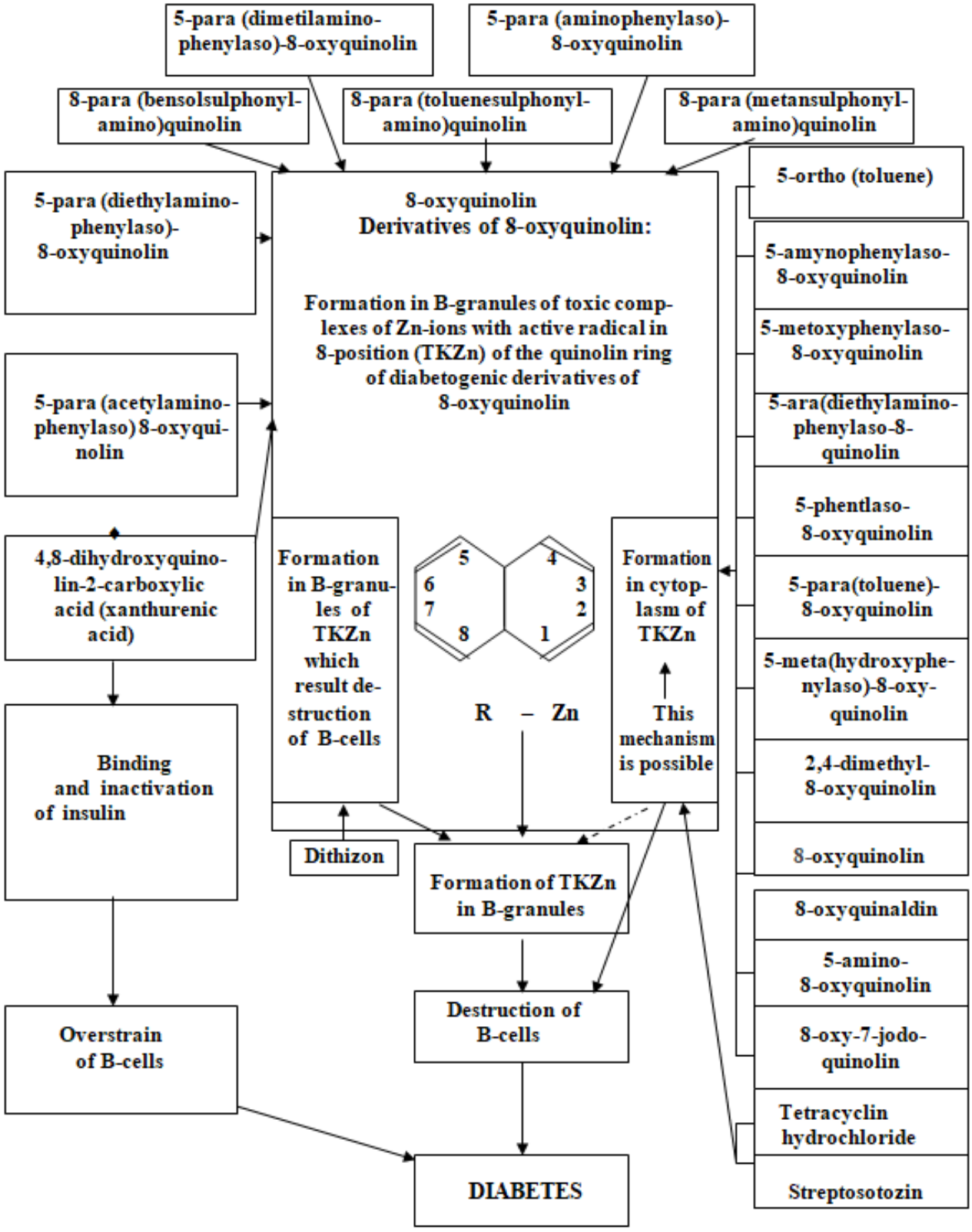
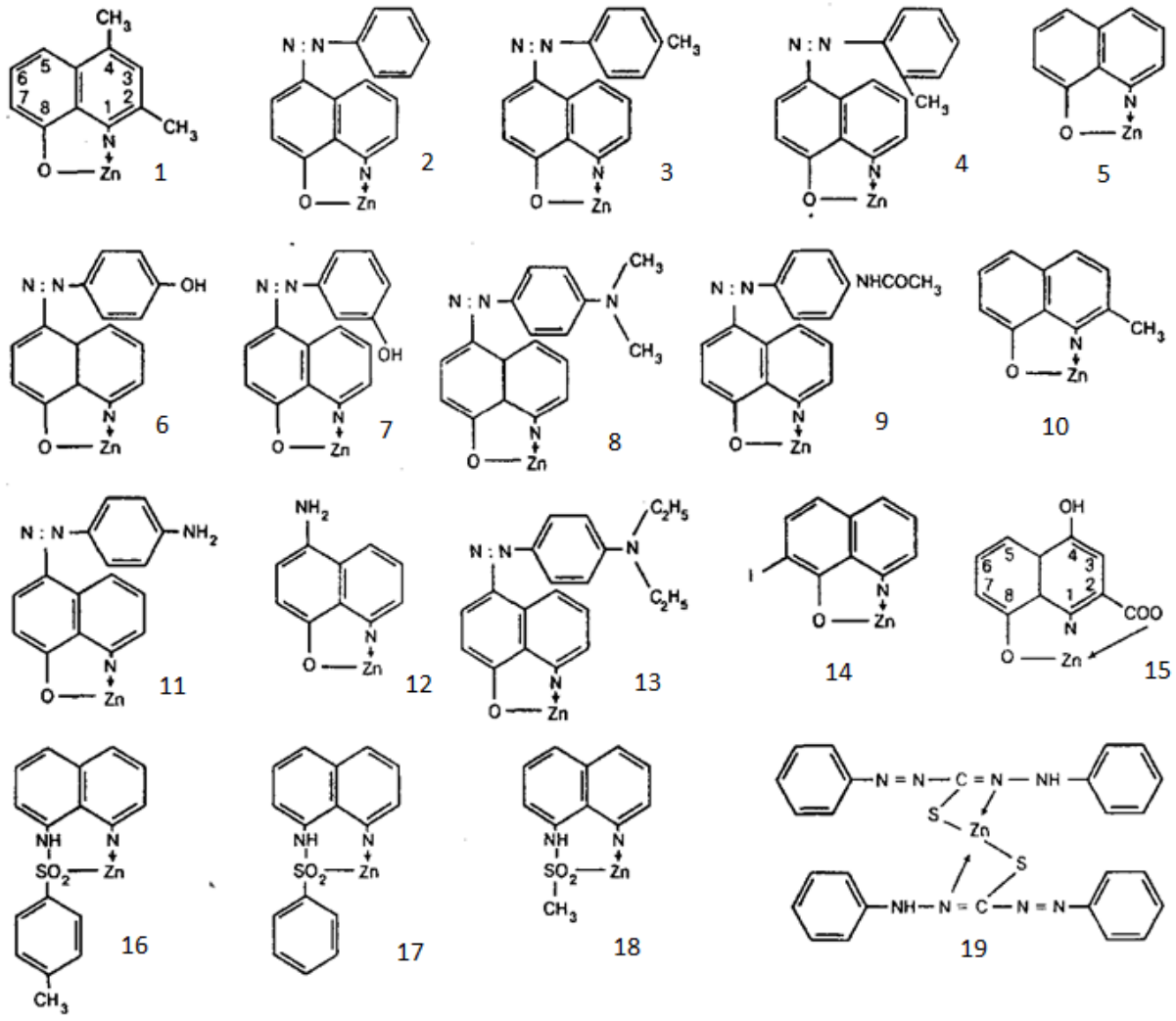


Figure 2. Mechanisms of damage of B-cells caused by diabetogenic chelat active chemicals (♦ — synthesized in human) (by Meyramova A.G. and Meyramov G.G., 2016)

Stability of complexes 1:1 formed by derivatives of 8-oxyquinoline is determined by a: 1) great number of double coupling in molecule of chelator; 2) forming of quadragonal ring; 3) derivatives of 8-arensulphonyl-aminoquinoline formed chelat-complex by aid of atom of S. Stability of the complex Zn-Xanthurenic acid is determined in added by fixation of the atom of Zn between 2 atom of O [9] (Fig. 3).



Compounds: *1* — 2,4-dimethyl-8-oxyquinoline, 35 mg/kg; *2* — 5-phenylazo-8-oxyquinolin, 20 mg/kg; *3* — 5-para(toluene)-8-oxyquinoline, 20 mg/kg; *4* — 5-orto(toluene)-8-oxyquinoline, 40 mg/kg; *5* — 8-oxyquinoline, 50–60 mg/kg; *6* — 5-para(diethylaminophenylazo)-8-oxyquinoline, 20 mg/kg; *7* — 5-meta(hydroxyphenylazo)-8-oxyquinoline, 30 mg/kg; *8* — 5-para(dimethylaminophenylazo)-8-oxyquinoline, 45 mg/kg; *9* — 5-para(acetylaminophenylaso)-8-oxyquinoline, 50 mg/kg; *10* — 8-oxyquinaldin, 10 mg/kg; *11* — 5-para(aminophenylazo)-8-oxyquinoline, 10 mg/kg; *12* — 5-amino-8-oxyquinoline, 30 mg/kg; *13* — 5-para(diethylaminophenylazo)-8-oxyquinoline, 40 mg/kg; *14* — 9-oxy-7-jodoquinoline, 50–60 mg/kg; *15* — 4,8-dihydroxyquinolin-2-carboxylic acid (xanthurenic acid); *16* — 8-para(toluenesulphonylamino)quinoline, 30–50 mg/kg; *17* — 8-(benzenesulphonylamino)quinoline, 30–100 mg/kg; *18* — 8-(metansulphonylamino)quinolin, 40–81 mg/kg; *19* — diphenylthiocarbazone (Dithizon), 45–50 mg/kg

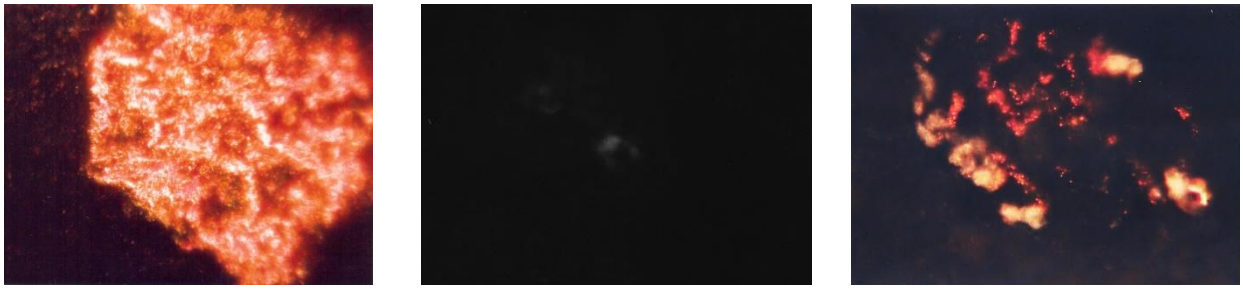
Figure 3. Complex salts of Diabetogenic zinc-binding chelate active chemicals with Zn-ions and its Diabetogenic doses (by Meyramova A.G., 2003)

Using of transmission electron microscopy method it was established that 2h past injection of Dithizon a strongly marked destruction of B-cells was developed: total devastation of cytoplasm of cell's matrix; destruction of mitochondria, endoplasmic reticulum and B-granules were discovered in the most parts of cells with remained matrix [7, 12]. Same results were obtained 1h later injection. Shortening of period since starting of injection showed that 15 min past injection in the contrary to 2h cell's matrix was remained on 80–90 % of B-cell's surface but 30–40 % appeared as zone free of matrix or zone of complete destruction of ultrastructures of B-cells [10]. Mechanisms of diabetogenic action of DZC are presented at Figure 2.

Chemical mechanisms of the methods for prevention developing of diabetes caused by chelators. Mechanisms of protective effect of aminoacids Gluthathione and Cystein

The aminoacids Glutathione and Cystein formed not toxic chelates with atoms of heavy metals due to sulfhydril radicals which have high affinity to ions of Zn^{+2}, Pb^{+2}, Cd^{+2} and Hg^{+2}. It is suggested that by these radicals aminoacids formed not toxic chelates with Zn-ions. The constant of stability of complex Zn-Glutathione is very high — 17.1–18.2.

Diabetes caused by DZC is prevented by Restored form of Glutathione (GRF). Preventive injection GRF,1000 mg/kg protect B-cells of rabbit's pancreas of binding of Zinc ions by DZ (Fig. 4) and from destruction and of developing of diabetes in all animals: normoglycemia and B-cells — without changes [14]. Meanwhile, oxydation of GRF result: two molecules of GRF formed one molecule with formation of disulfide connection (Fig. 5). Thus, oxidized form of glutathione (GOF) have same structure but contrary to GRF not contain in structure of molecule of SH-radical not protect B-cells of formation of complex Zn-DZ that result destruction of cells. Injection to animals of 1000 mg/kg of GOF not protect B-cells of destruction by DZC and diabetes developed in all animals [15, 16].



1 2 3

1 — Negative fluorescent reaction for Zinc in B-cells (absence of fluorescence) as result of binding of Zinc with GRF; high specific for Zinc reaction with 8PTSQ; ×140; *2* — Injection of GRF and *3* — 10 min later of DZ; prevention of formation of complex Zn-DZ as result of blockingof Zinc by GRF; darc microscopy; ×280

Figure 4. Red granules of complex Zn-DZ in B-cells of rabbit; staining by DZ; darc microscopy; ×280

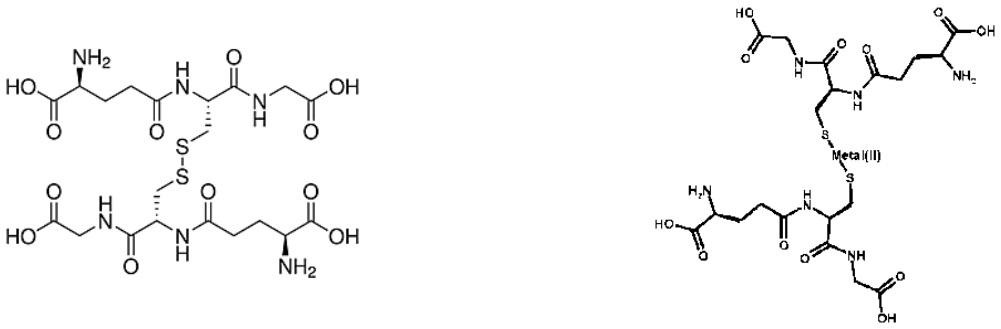


Figure 5. Disposition of Zinc atom between 2 atoms of S of two SH-groups from 2 molecules of GRF (by F.M. Rubino. Toxicity of Glutathione-Binding Metals: A Review of Targets and Mechanisms. Toxics, 2015, 3(1), 20–62)

The GRF easily reacts with free radicals among which it should be noted hydroxylic and carbon radicals, giving Hydrogenium atom. Similar interactions provide protection, neutralizing the fissile OH^- radical which is considered as the most dangerous among the free radicals. Decrease of amount of GRF increases susceptibility of animals to cytotoxins [17]. SH-radical possess chemical resistance against influence of peptidases.

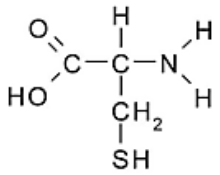
Its polygonality determined by chemical properties and allows to be simultaneous both the nucleophilic agent and the fissile reducer, interacting with numerous elektrofilny and oxidizing components, such as N_2O_2, O_2 and OH^-. GRF as active reducer plays an important role in processes of a detoxification.

Glutathione is used for prevention and treatment of diabetic neuropathy in the streptozotocin-induced diabetic rat [18]. It was supposed that inactivation or change of SH-group of sulfhydril radicals in molecules

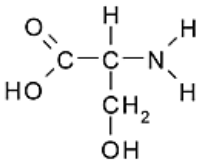
of Glutathione result complete disappearing of protective properties of the formed in result of Oxidized form of Glutathione.

It is evidently easy and clear to suppose that preventive effect Restored form of Glutathione is determined by inactivation of SH-radicals of two molecules of GRF and fixation of atom of Zinc between two atoms of S to which Zinc possess a high affinity (Fig. 5).

Injection of Cystein, 1000 mg/kg prevent formation in B-cells of toxicchelat Zn-DZ an complete prevention of diabetes in all animals within 6 h; 12 h past injection diabetes was prevented in 6 animals from 8 and 24 h past injection of Cystein — in 2 animals from 4. Cystein protect B-cells of destruction caused by diabetogenic derivatives of 8-oxyquinoline [19]. Aminoacid Serin, which contains hydroxyl radical in molecule instead of sulfhydril radical in molecule of Cystein, not possess diabetogenic properties.



Cystein



Serin

Aminoacid Hystidine formed with Zn-ions high stable complex 2:1 which logarithm of constant of stability is 12.0. Contrary toother aminoacids chelat activity of Hystidin is determined by the presence in molecule of the imidazol ring [8]. Injection to animals 1000 mg/kg of the Hystidin Hydrochloride (HH) result complete prevention of diabetes past injection of Dithizon followed 5 min past injection of HH and — in half of total number of animals injected of Dithizon 0.5–1 h past injection of HH [20].

Chemical mechanisms of protective effect of derivatives of Dithiocarbamic acid

Derivatives of Diethyldithiocarbamic acid (DDC) possess a high affinity for Zinc ions as EDTA were conducted. Na salt of DDC is able not only to prevent developing of diabetes caused by DZ but to displace of DZ from formed in B-cells complexes as Zinc-DZ due to more high affinity to Zinc. EDTA as chelator possess more high affinity to Zn and constant of stability of its chelats with Zn is 13.1 meanwhile with ions of Mg^{+2}, Ca^{+2} and Fe^{+3} correspondly 5.4, 7.3, and 10.9 (10). It was showed that EDTA prevent diabetogenic action of streptosotocin by binding of Zn-ions. More detail investigation of processes of interaction of Zn-ions contained in B-cells with NaDDC showed that injection of 1000 mg/kg to rabbits result complete binding of all amount of Zn-ions in B-cells that accompanied by formatuion in B-cells of not toxic chelate complexes 2:1 as Zinc-NaDDC (10) (Fig. 6). Atom of Zinc is fixed between of two atoms of S from the two molecules of NaDDC. Followed injection of DZ not accompanied by formation of toxic Zn-DZ complex in cytoplasm of B-cells and diabetes not developed. Thus, finally it was confirmed that presence of toxicchelat complexes of DZ and diabetogenic derivatives of 8-oxyquinoline in B-cells within first 15–30 min after its forming result not visible for the first a few hours incorrigible destructive changes in B-cells. Formed more later degenerative histological changes in islets is result of action of chelators in the first 15 min.

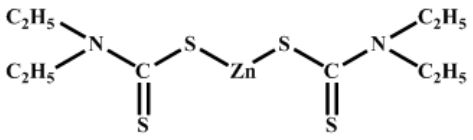


Figure 6. Fixation of atom of Zinc between two atoms of S of the two molecules of NaDDC

It is known that Streptozotocin possess chelate properties and have high affinity to Zn-ions. Alterative action of Streptozotocin may be prevented or eased by preventive action of EDTA [21].

Investigation of diabetogenic properties of Dithizon and derivatives of 8-oxyquinoline have theoretical significance because these chemicals are not formed in human and really. In added peroral administration of its is not effective because they are not soluble and not absorbed in intestinum. Parenteral injection of diabetogenicchelators result developing of diabetes only. Solutions of all these chelators are not stable and only injection of the fresh prepared solutions (ex tempore) result diabetogenic effect. Meanwhile some antimicrobial drugs widely used for treatment of skin diseases contains derivatives of 8-oxyquinolin as main antimicrobial component.

Among 18 diabetogenic derivatives of 8-oxyquinoline the Xanturenic acid (XA) only is formed in animals and elderly humans in deficiency of Pyridoxine. It is known that XA is accumulated in organism of old human as result of disturbances of Tryptophan metabolism. Low doses of the XA accumulated in human gradually. May be that is why diabetes caused by XA developed gradually as type 2 in opposite to type 1 diabetes caused by injection of diabetogenic doses of other chelators. High concentration of XA in the urine decrease by long time prolonged using of Pyridoxine [22] that accompanied by decreasing of blood glucose concentration as weakening of symptoms of diabetes.

The number of diabetogenic chelators human have contacts is increased year by year. As example Tetracycline hydrochloride is active chelator which have high affinity to Zn-ions and formed with it complex 1:1 and 2:1 with high constant of stability as 9.0 [11]. Direct action on B-cells of high doses of tetracycline result hyperplasia and degeneration of cells. Isoniazid, a drug for treatment of tuberculosis, formed pentagonal stable chelats with Zn-ions. May be more high frequency of diabetes among patients treated by Isoniazid determined by this fact? This interest is increased taking into consideration fact that in this case concentration of the Xanturenic acid in urine is high because Isoniazid in antagonist of Pyridoxal-5-Phosphate.

Dehydroascorbic Acid (DA) which is formed me symptoms of diabetes on animals as of solucose level id in organism as result of metabolisation of Ascorbic Acid, possess diabetogenic properties and result direct alterative effect on B-cells. Concentration of DA in organism of diabetics is evidently increased in opposite to decreasing concentration of Ascorbic acid.

It is known that chelators which formed with Zn-ions tetragonal or pentagonal rings possess diabetogenic properties. Chelators contains in molecule as least 4 or 5 double chemical connections possess diabetogenic properties also in opposite tochelators contained 1–2 or not contained its which not possess analogical properties. As example — derivatives of Diethyldithiocarbamic acid of Dimethyldithiocarbamic acid, aminoacids Cystein, Glutathione and Hystidine. Complexes formed by noted above protectors not contains in molecule tetragonal or pentagonal rings and not containsor contains minimal number (1–2) of double connections. Administration of large amount of these chelators not result destruction of B-cells and protect, in opposite, B-cells of destruction caused by diabetogenicchelators.

Noted above data put us to look on these chemicals as on one possible factor in ethiology of human diabetes. The significance of this possibility is increased taking consideration fact that human pancreas contains large amount of Zn-ions possess to form chelat complexes with diabetogenic chelators.

Obtained results demonstrated that Glutathione reduced form's protective activity determined by its ability to prevent formation of toxic chelate complexes with DZC due to high affinity for Zinc and more suitable for to elaborate of methods for prevention of diabetes caused by DZC synthezised in human.

Conclusions

1. Diabetogenic zinc binding chemicals formed toxic for cells intracellular chelate salts with zinc in pancreatic B-cells by fixation of zinc atom between oxygen and nitrogen atoms, between two atoms of oxygen or between sulfur and nitrogen atoms due to high affinity of zinc for high affinity of zinc in relation to these chemical elements.

2. Non-diabetogenic zinc binders as derivatives of Dithiocarbamic acid, as well as amino acids — a Restored form of Glutathione and Cysteine formed intra-complex salts with zinc by fixation of zinc between two sulfur atoms of two molecules of the aminoacids in all cases; such complexes do not cause damage and death of B-cells, preserving their function.

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Диабетогенді уытты заттардың ұйқы безінің мырышымен өзара әрекеттесуінің химиялық механизмдері және оның алдын алу әдістері

Әдебиетке шолуда соңғы онжылдықта адаммен байланысу мүмкіндігі біртіндеп артып, химиялық мырышбайланыстырушы заттар туындататын экспериментальды диабеттің дамуын алдын алу әдістері туралы деректер келтірілген. Олардың арасында, негізгі назар бұрында маңыздылығына азырақ көңіл бөлінген глютатионның аминқышқылды топтың алдын алу бейімділік қабілеті бар екендігі ескерілген. Мақалада көрсетілгендей, глютатионның мырышқа деген жоғарғы белсенділігі оның құрылымында SH-молекула топтың болуымен, осы арқылы диабетогенді емес мырыштың бұғатталуы диабетогенді хелаторлармен байланысын туындатпайды, нәтижесінде инсулин өндіруші В-жасушалары 15–30 минут шамада жойылады. Сондай-ақ, диабетогенді мырышбайланыстырушы заттар (ДМЗ) туындататын диабеттің дамуына жол бермейтін, молекула құрылымында сульфгидрильді топтардан тұратын тағы екі аминқышқылдың мүмкіндігі туралы деректер берілген. Әдебиетке шолуда мырышқа қатысты кешенді қалыптастырушы қасиеттері бар кейбір дәрілік препараттар туралы ақпараттар келтірілген.

Кілт сөздер: мырыш, В-жасушалар, диабетогенді мырышбайланыстырушы хелаттар, глютатион, дифенилтиокарбазон (дитизон).

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Химические механизмы взаимодействия диабетогенных токсических веществ с цинком поджелудочной железы и методы его предотвращения

В обзорной статье приведены данные о методах предотвращения развития экспериментального диабета, вызываемого химическими цинксвязывающими веществами (ДЦВ). На основе данных многолетних исследований (1964–2018) авторами детально проанализированы и обоснованы химические механизмы блокирования цинка в клетках, благодаря чему предотвращается возможность их разрушения диабетогенными цинксвязывающими веществами. По результатам собственных исследований и данных литературы изучены химические механизмы предупреждающего действия производных дитиокарбаминовой кислоты, аминокислот — восстановленного глютатиона, цистеина и гистидина, а также возможности химической нейтрализации в крови ДЦС до того, как они достигнут поджелудочной железы.

Ключевые слова: цинк, В-клетки, диабетогенные цинксвязывающие вещества, глютатион, дифенилтиокарбазон (дитизон).

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