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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

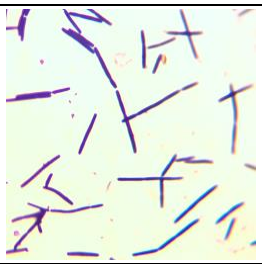
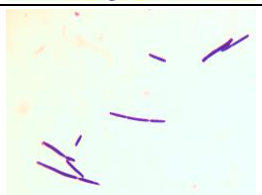
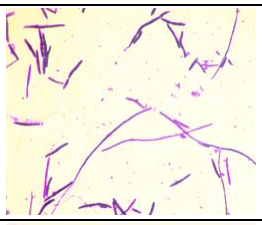
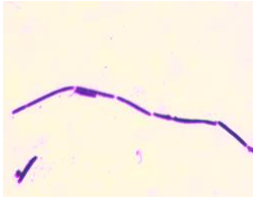
of lactobacteria cells. Inoculated plates with discs were incubated at 37±1 °C for 48 h. The antibiotic graph was formed by the diameter of the growth retardation zone of microorganisms. The study was performed in triplicate and the results were expressed as arithmetic mean.

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

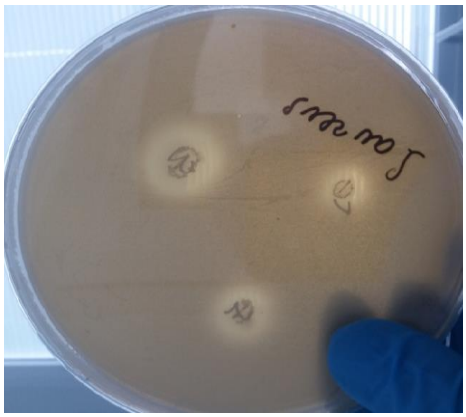


Figure 1. Antagonistic activity of *Lactobacillus* isolates against test-strains *Staphylococcus aureus* NCTC 12973/ ATCC® 29213™

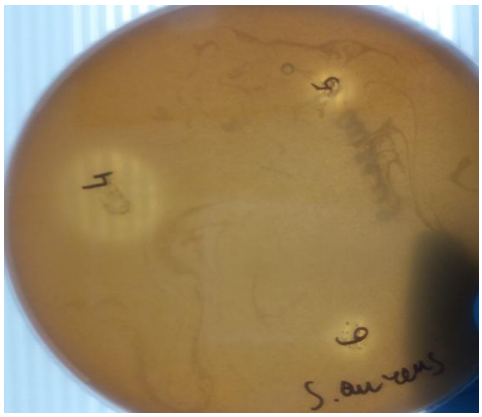


Figure 2. Antagonistic activity of *Lactobacillus* isolates against test-strains *Staphylococcus aureus* NCTC 12973/ ATCC® 29213™

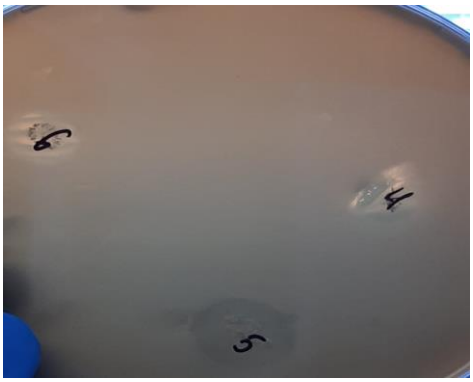


Figure 3. Antagonistic activity of *Lactobacillus* isolates against test-strains *Salmonella typhimurium* NCTC 12023/ ATCC® 14028™

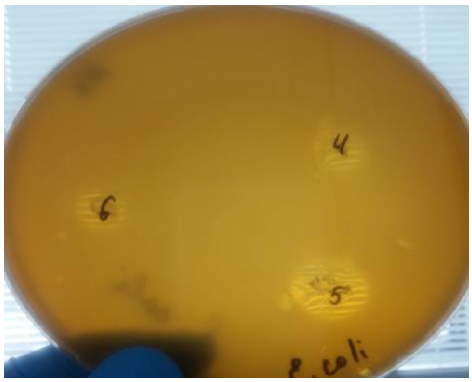


Figure 4. Antagonistic activity of *Lactobacillus* isolates against test-strains *Escherichia coli* NCTC 12923/ ATCC® 8739™

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

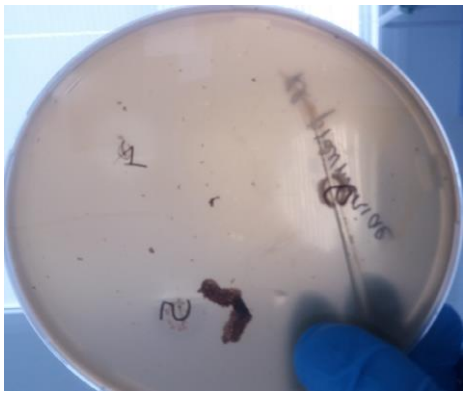


Figure 6. Antagonistic activity of *Lactobacillus* isolates against test-strains *Klebsiella pneumonia* NCTC 9633/ ATCC® 13883™

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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