

Determination of Antagonistic Activity and Antibiotic Resistance of Bacteria and Yeast for Starter Cultures: An Experimental Study to Increase the Production of Fermented Milk Drinks in Kazakhstan

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Abstract

The interest in the study of lactic acid bacteria is due not only to the fact that they play a large and versatile role in human life. Researchers pay special attention to their biological properties, such as adhesiveness, antagonistic, immunoregulatory, antitumor, cytoprotective, and cholesterol-neutralizing activity, phage resistance, and bacteriocinogenity. This entails the need to constantly isolate new species of lactic acid bacteria and their phages, study their characteristics and systematic position in modern classification, and expand the ways of their practical application in the food industry. The purpose of the paper is to present the results of the antagonistic activity and antibiotic resistance of lactic acid bacteria and lactic acid bacteria yeast, which form the basis of priority starter cultures in the production of fermented milk drinks. To conduct the study, we obtained 56 strains of lactic acid bacteria newly isolated from products whose production was located in three geographically distributed regions of Kazakhstan. The media for maintaining isolated cultures of lactic acid bacteria and yeast were De Man–Rogosa–Sharpe (MRS) agar, milk hydrolysate (MH) (according to Bogdanov), MH with agar, generally accepted diagnostic media, and wort agar. The optimal environment for the manifestation of antagonistic properties is the MRS agar. The studied strains are sensitive mainly to ampicillin, levomycetin, and streptomycin, and to the rest of the antibiotics, they are slightly sensitive or not sensitive at all.

Keywords

Kumis, Shubat, Yeast, Strain, Milk

Introduction

Lactic acid bacteria are a morphologically heterogeneous group of microorganisms widely used in the national economy. Lactic acid bacteria are used as starter cultures in the production of various fermented dairy products for the following reasons: the ability to form lactic and acetic acids [1], aromatic compounds, and polysaccharides that give a specific taste and structure [2]; the presence of an antibiotic, antitumor and antileukemic activity, which can positively affect human health [3-6], a metabolic activity which increases the digestibility of fermented milk products, growth in a wide temperature range and various combinations, which makes it possible to produce a variety of fermented milk products [7]. Lactic acid bacteria are widely used in starter cultures in the production of various fermented products [8, 9]. One of the main reasons is that some lactic acid bacteria have an ability that most other microorganisms do not have: they can use milk sugar (lactose), and in this, they are similar to many intestinal bacteria. Due to the formation of large amounts of lactic acid, to which they are largely tolerant, lactic acid bacteria, under suitable conditions, can multiply quite

quickly, expelling other microorganisms.

Lactic acid bacteria are widespread in the biosphere and under the influence of anthropogenic and technology-driven factors, their total number in the biosphere is continuously increasing. The place of their natural habitat, as the results of the study show, can be the soil and rhizosphere of plants, the water of lakes and rivers, their sand ridges, and the digestive tract of animals and humans [10].

The isolation and quantitative accounting of lactic acid bacteria in natural and industrial substrates is associated with some difficulties. This is because representatives of this group of microorganisms are very demanding of food sources and do not grow on simple media. Most of them need some vitamins (lactoflavin, thiamine, pantothenic, nicotinic, and folic acids, biotin) [11] and amino acids, as well as purines and pyrimidines [12]. Their cultivation requires complex media containing vegetable broths, meat and yeast extracts, whey, and protein hydrolysates. The study of the behavior of microorganisms in various media is actively continuing. It has been shown that some lactic acid bacteria, when growing on media containing blood, form cytochromes and may even be able to perform phosphorylation in the respiratory chain [13].

In recent years, a group of lactic acid bacteria has provided essential living reagents in studies of vitamins and amino acids. They are used not only in the work of scientific laboratories but also in the production practice of the pharmaceutical and food industries.

By the shape of the cells, lactic acid bacteria are classified into rods and cocci. Their sizes vary in individual species.

The energy source for these bacteria is lactic acid fermentation, where adenosine triphosphate is formed during the anaerobic oxidation of organic substrates during substrate phosphorylation reactions [14]. Concerning oxygen, they occupy an intermediate position between obligate anaerobes and cytochrome-containing facultative and obligate aerobes. Most lactic acid bacteria are in dire need of the presence of several amino acids, various vitamins, and biologically active substances in the environment. The need for them varies greatly among individual species and strains.

Depending on the type of fermentation products, they are divided into two groups: homofermentative and heterofermentative.

Lactic acid bacteria belong to the genera *Lactobacillus*, *Leuconostoc*, *Streptococcus*, and *Pediococcus*.

The *Lactobacillus* genus includes rod-shaped bacteria, among which homo- and heterofermentative species are found. The fermentation product is D(-) lactic acid (*Lactobacillus bulgaricus*) and DL(±) lactic acid (*Lactobacillus acidophilus*). The peculiarity of *L. bulgaricus* and *L. acidophilus* is the ability to produce antibiotic substances that suppress the development of intestinal microflora. The greater resistance of *L. acidophilus* to adverse environmental conditions compared with *L. bulgaricus* (growth at pH 8.0; presence of 20% bile in the medium; 2% sodium chloride, and phenol) expands the range of use of this culture in terms of obtaining fermented dairy products for

specialized purposes and lactic acid.

Lactobacilli are Gram-positive, and catalase-negative, as a rule immobile, do not form spores, usually do not restore nitrate, utilize glucose enzymatically, and have proteolytic activity. The *Lactobacillus* genus includes over 50 species and subspecies, the content of G+C pairs in DNA ranges from 32 to 55% [15].

From the *Lactobacillus* genus, atypical Lactobacilli are classified into a separate *Carnobacterium* genus. The species *L. divergens* and *L. piscicola* were transferred to this genus and two more new species were included.

The status of some *Lactobacillus* species has been changed. Several previously independent species have been reclassified as subspecies: *L. bulgaricus* as *L. delbrueckii* subsp. *bulgaricus*, *L. lactis* as *L. delbrueckii* subsp. *lactis*, etc. On the contrary, *Lactobacillus casei* subsp. *rhamnosus* received the status of an independent species, *L. rhamnosus*. Some species are recognized as synonyms of other species: *L. cellobiosus* is considered a synonym of *L. fermentum*, and *L. Leichmani* is a synonym of *L. delbrueckii* subsp. *lactis* [16].

In recent years, the consumption of dairy and fermented milk products has increased significantly worldwide, and these products are increasingly being used as therapeutic agents for the treatment of various diseases [16]. This is primarily because, in the past, the enzymatic process in milk was caused by unpredictably slow fermenting microorganisms. In turn, milk can affect the spread of infectious diseases. The importance of milk as a source of infectious diseases [17] is associated with the ability of pathogenic microorganisms not only to persist but in most cases to multiply in this environment. Thus, the causative agents of dysentery *sonnei* multiply in milk, so the course of dysentery is often similar to the type of food toxic infection. Since the intensity of reproduction of pathogenic microorganisms in milk and dairy products depends on the antagonistic action of lactic acid bacteria, the selection of antagonistic bacteria for starter cultures plays an important role.

Therefore, research on the isolation and study of new lactic acid cultures with high antagonistic activity is important [18, 19].

The quality of the resulting lactic acid product largely depends on the composition of the starter culture used. Each group of organisms contributes to the process of its preparation. Metabolites of some types of lactic acid bacteria give flavor and bouquet to the finished product, others affect the taste, some accelerate the process of peptonization, giving a certain consistency to the product, and yeast enriches the medium with a complex of vitamins [20].

Co-cultivation with yeast allows for maintaining the viability of lactic acid bacteria for a long time. The protective effect of yeast does not depend on its ability to form alcohol but may depend on changes in the pH of the medium due to the formation of alkaline products, changes in the state of the protein part of milk as a result of proteolysis, which is caused by yeast in co-development with lactic acid bacteria; and the release of enzymes or vitamins [21].

Modern industrial food technologies use specific dairy microorganisms, which allows for specific fermentation under specified conditions, creating fermented milk products with high nutritional, physicochemical, sanitary, and medicinal properties. Lactic acid bacteria also help to increase their digestibility. The nutritional needs of lactic acid bacteria vary depending on the strain and cultivation conditions, so it should be determined on a case-by-case basis.

In Kazakhstan, leveraging biotechnological advancements for the complex and efficient processing of agricultural raw materials into food products is a growing trend [22]. This is particularly relevant for creating new dairy-based products and developing technologies to produce nutritious options for populations in environmentally challenged regions or those prone to gastrointestinal health issues.

The production of traditional fermented milk drinks like shubat and kumis has seen significant growth, with increases of 5.7% and 52.8% respectively, from January to September 2023 compared to the same period in 2022 [22]. This surge in fermented milk drink production is not only preserving cultural heritage but also contributing to food security by providing a sustainable source of nutrition. Enhanced production techniques and the development of starter cultures are crucial for expanding the availability of these nutritious beverages.

Research into the microflora of traditional drinks such as kumis, shubat, and ayran is essential for isolating beneficial lactic acid bacteria and yeast, which can lead to the creation of superior starter cultures. Our work focuses on studying the lactic acid flora of these dairy products, aiming to isolate new strains of lactic acid bacteria, preserve existing strains, and develop bio-preparations. These endeavors are intended to support the manufacture of high-quality fermented milk products, thus ensuring a resilient food system that contributes to the national food security strategy by increasing the availability of nutritious, locally produced foods.

The purpose of the paper is to present the results of the antagonistic activity and antibiotic resistance of lactic acid bacteria and yeast of lactic acid bacteria, which form the basis of priority starter cultures in the production of dairy products.

Materials and Methods

The objects of the study were 56 strains of lactic acid bacteria newly isolated from products from various regions of Kazakhstan, namely, Almaty, Kyzylorda, and Karaganda.

New strains of lactic acid bacteria were selected from the following sources:

- Non-pasteurized cow's milk and mare's milk.
- Homemade ayran.
- Kumis.
- Shubat.
- Curdled milk.

The reasons for the choice were conditioned by the fact that these food products are actively produced and consumed

in Kazakhstan. These are the gentlest natural dietary foods with many medicinal properties, so the range of their use is very extensive. They are periodically included in diets in small quantities for almost any disease.

Nutrient media

The main media for maintaining isolated cultures of lactic acid bacteria and yeast were the following:

- **MRS agar:** Yeast autolysate: 5 ml, peptone: 10 g, glucose: 20 g, ammonium citric acid: 2 g, sodium acetic acid: 5 g, $\text{MgSO}_{4.7}\text{H}_2\text{O}$: 200 g, $\text{MnSO}_{4.4}\text{H}_2\text{O}$: 50 mg, K_2PO_4 : 2 g, tween 80: 1 ml, agar-agar: 20 g, water: 1 L, pH 6.2 - 6.6 [23].
- **MH:** According to Bogdanov; to prepare it, 1 g of pan-creatin and 5 ml of chloroform were added to 1 liter of boiled and cooled sterile skimmed milk (pH = 7.4 - 7.6). The vessel was closed with a cork stopper and placed in a thermostat for 72 h at 40 °C. The resulting hydrolysate was filtered, pH 7.0 - 7.2 was set, and the hydrolysate was sterilized for 10 min, at 1 atm [24].
- **MH with agar:** When preparing the agarized medium, the MH was diluted twice with water and 2% of agar-agar was added.
- **Generally accepted diagnostic media:** Gelatin dilution medium, media with various carbohydrates, medium for the formation of ammonia from arginine, SM with methylene blue 0.1 and 0.3%, MH with 4.5 and 6.5% sodium chloride, medium for determining the formation of gas from glucose.
- **Wort agar.**

Isolation of lactic acid bacteria

The isolation of lactic acid bacteria and yeast was carried out according to figure 1.

The grown colonies of presumably lactic acid bacteria were studied under a microscope and seeded into test tubes

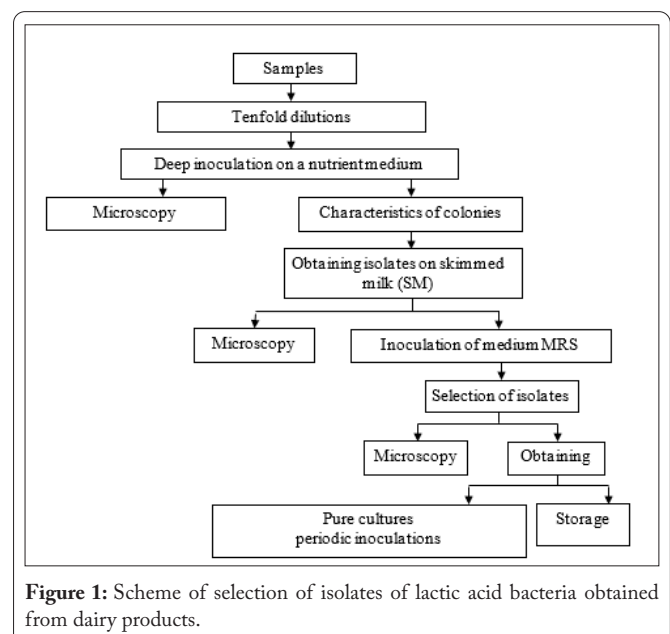


Figure 1: Scheme of selection of isolates of lactic acid bacteria obtained from dairy products.

with sterile liquid nutrient media (MH, MRS medium). The tubes were cultured in thermostats at 30 °C and 37 °C for 24 h and used in further studies.

The antagonistic activity of bacteria and yeast was studied by diffusion into agar. Gram-negative bacteria (*Escherichia coli*, *Salmonella dublin*) and Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Sarcina flava*, *Mycobacterium rubrum*, *Mycobacterium citreum*) were used as test cultures. The test cultures were grown on media with an optimal composition for each species: meat and peptone agar Gause's medium number 2.

0.1 ml of suspension was introduced into the molten and cooled medium, mixed, and poured into a Petri dish. Wells were cut out in the nutrient medium layer, into which daily cultures of the studied lactic acid bacteria were introduced and placed in a thermostat for 24 h. A day later, the growth suppression zones of test cultures with lactic acid bacteria were measured.

Observations were conducted daily for 3 days at 28 °C and 37 °C. To identify the strongest antagonistic activity, optimal conditions for the cultivation of lactic acid bacteria were selected by growing on various nutrient media (MH and MRS medium).

To study antibiotic resistance, we used the generally accepted method of standard indicator discs impregnated with standard solutions at a concentration of 0.1 ml of streptomycin, levomycitin, ampicillin, benzylpenicillin, erythromycin, vancomycin, gentamicin, and tetracycline (Agat-Med LLC, Balashikha, Russia). MH with agar was used as a nutrient medium for lactic acid bacteria. In the experiments, single-day cultures grown at an optimal temperature were used in the form of a suspension of cells in the amount of 1 billion/ml (according to the bacterial turbidity standard), based on the calculation of 0.1 ml of suspension per Petri dish. After inoculating the dishes with the studied cultures of the lactic acid bacteria, discs containing antibiotics were placed on the surface of the nutrient medium. Cultivation was carried out for 3 days at 28 °C and 37 °C. The sensitivity of lactic acid bacteria to antibiotics was determined by measuring the growth suppression zone.

To obtain reliable results, all experiments were carried out in 3 - 5 repetitions.

Results and Discussion

Screening of antagonist strains

The formation of therapeutic, dietary, and preventive qualities of dairy products is achieved not only by the balance of their components, which determine the biological value but also by the selection of lactic acid cultures in the microflora of the starter culture. Unlike other food products, fermented dairy products contain, along with valuable nutrients, a huge number of living cells of microorganisms (Lactobacilli), up to 10⁹ cells in the product. In percentage terms, living cells make up 1 - 2% of the mass of the product. These bacteria and the metabolic products they produce cause a more subtle specific effect of fermented milk products on the human body and additional individual medicinal properties of a particular product [25].

One of the essential properties of certain types of microorganisms and their combinations is to inhibit and suppress the development of undesirable and even dangerous to human health mesophilic bacteria of the *Enterobacteriaceae* family of the genera *Escherichia*, *Proteus*, and *Salmonella* [26].

As a rule, cultures with pronounced antagonistic properties are included in the composition of starter cultures. Therefore, the objectives of our research included the study of the antagonistic activity of lactic acid bacteria and yeast during their growth on various nutrient media.

The initial selection of bacteria isolated from dairy products was carried out to include in the starter culture the determination of antagonistic activity against conditionally pathogenic strains of *E. coli* and *S. aureus*.

A total of 56 microorganisms were studied (Table 1). From the results, 21 strains of lactic acid bacteria and yeast were selected for further research and tested as antagonists against seven test cultures of Gram-positive and Gram-negative microorganisms (*B. subtilis*, *S. flava*, *E. coli*, *S. aureus*, *S. dublin*, *M. rubrum*, and *M. citreum*).

Antagonistic activity in most cultures manifests itself after 24 h and in some increases by 48 h of cultivation.

Figure 2 shows the results of the antagonistic activity of *Lactococcus* subsp. *lactis* and *L. lactis* subsp. *cremoris* species grown on.

Lactococcus subsp. *cremoris* inhibits the growth of *E. coli*, *B. subtilis*, and *S. aureus* to the greatest extent, and the growth of *S. dublin* and *S. flava* to the least extent. In *Lactococcus* subsp. *lactis*, antagonism is most pronounced against *E. coli*, *B. subtilis*, and *S. aureus* (12 - 23 mm). Concerning *M. rubrum* and *M. citreum*, all strains have a pronounced degree of antagonistic activity.

The antagonistic activity of *Lactobacillus* cultures isolated from various regions was also studied (Figure 3). Quite strong antagonists against *E. coli* have been found among *L. acidophilus* strains. *L. Acidophilus* gives the largest zone of growth retardation of mycobacteria and inhibits *B. subtilis*. *L. bulgaricus* cultures gave a *S. dublin* inhibition zone of 7 - 13 mm. The activity range of *L. acidophilus* cultures was in the range of 10 - 23 mm.

Yeast *Torulopsis kefir* var. *kumis* inhibits Mycobacteria, *E. coli*, and *B. subtilis* (17 - 21 mm) (Figure 4).

The spectrum of antimicrobial action of *Lactococci* is comparable to that of lactobacilli. The strains inhibited the growth of both Gram-positive bacteria, including facultative anaerobic cocci *S. aureus*, aerobic coryneform bacteria *M. rubrum* and *M. citreum*, spores *B. subtilis*, and Gram-positive facultative anaerobic rods *E. coli*.

Antagonistic activity of some strains isolated by us, *L. lactis* subsp. *lactis* K-8 and *L. lactis* subsp. *lactis* BT-5; *L. acidophilus* BB-15, *L. acidophilus* M-3, *L. acidophilus* EB-23 *L. bulgaricus* GM-8 and *T. kefir* var. *kumis* 23, can be evaluated as quite high.

Table 1: Antagonistic activity of bacteria isolated from dairy products.

No.	Strain species	Growth inhibition zone (mm)	
		<i>E. coli</i>	<i>S. aureus</i>
1	<i>L. lactis</i> subsp. <i>lactis</i> K-8	23 ± 1.5	21 ± 1.3
2	<i>L. lactis</i> subsp. <i>lactis</i> BT-5	21 ± 1.3	16 ± 0.2
3	<i>L. lactis</i> subsp. <i>lactis</i> BK-3	11 ± 0.3	8 ± 0.3
4	<i>L. lactis</i> subsp. <i>lactis</i> ShS-10	11 ± 0.3	8 ± 0.3
5	<i>L. lactis</i> subsp. <i>lactis</i> MSh-23	18 ± 0.8	19 ± 0.8
6	<i>L. lactis</i> subsp. <i>lactis</i> SP-3	6 ± 0.1	4 ± 0.1
7	<i>L. lactis</i> subsp. <i>lactis</i> KOM-2	12 ± 0.4	10 ± 0.4
8	<i>L. lactis</i> subsp. <i>lactis</i> Sh-4	9 ± 0.3	10 ± 0.4
9	<i>L. lactis</i> subsp. <i>lactis</i> ZhI-9	11 ± 0.3	7 ± 0.2
10	<i>L. lactis</i> subsp. <i>lactis</i> TT-4	16 ± 0.5	18 ± 0.4
11	<i>L. lactis</i> subsp. <i>lactis</i> S-12	16 ± 0.5	14 ± 0.4
12	<i>L. lactis</i> subsp. <i>lactis</i> TS-26	16 ± 0.5	18 ± 0.4
13	<i>L. lactis</i> subsp. <i>lactis</i> KK-7	18 ± 0.4	16 ± 0.5
14	<i>L. lactis</i> subsp. <i>lactis</i> MR-7	8 ± 0.3	12 ± 0.4
15	<i>L. lactis</i> subsp. <i>lactis</i> GR-7	10 ± 0.4	10 ± 0.4
16	<i>L. lactis</i> subsp. <i>cremoris</i> AS-5	10 ± 0.4	13 ± 0.3
17	<i>L. lactis</i> subsp. <i>cremoris</i> ChS-11	10 ± 0.4	15 ± 0.2
18	<i>L. lactis</i> subsp. <i>cremoris</i> Zh-3	12 ± 0.3	14 ± 0.2
19	<i>L. lactis</i> subsp. <i>cremoris</i> GZh-31	12 ± 0.4	8 ± 0.3
20	<i>L. lactis</i> subsp. <i>cremoris</i> TYu-7	15 ± 0.2	7 ± 0.2
21	<i>L. lactis</i> subsp. <i>cremoris</i> D-11	5 ± 0.1	11 ± 0.3
22	<i>L. lactis</i> subsp. <i>cremoris</i> OD-11	14 ± 0.4	17 ± 0.6
23	<i>L. lactis</i> subsp. <i>cremoris</i> U-4	16 ± 0.6	19 ± 0.8
24	<i>L. lactis</i> subsp. <i>cremoris</i> TM-5	18 ± 0.8	18 ± 0.8
25	<i>L. lactis</i> subsp. <i>cremoris</i> AR-2	11 ± 0.3	12 ± 0.3
26	<i>L. acidophilus</i> GK-5	8 ± 0.2	5 ± 0.1
27	<i>L. acidophilus</i> ZhT-1	16 ± 0.5	17 ± 0.5
28	<i>L. acidophilus</i> EB-23	18 ± 0.8	19 ± 0.9
29	<i>L. acidophilus</i> AB-18	14 ± 0.4	16 ± 0.5
30	<i>L. acidophilus</i> BB-15	22 ± 1.4	20 ± 1.4
31	<i>L. acidophilus</i> B-52	18 ± 0.7	17 ± 0.7
32	<i>L. acidophilus</i> B-7	15 ± 0.4	10 ± 0.03
33	<i>L. acidophilus</i> SM-2	13 ± 0.3	13 ± 0.3
34	<i>L. acidophilus</i> M-3	20 ± 1.4	23 ± 1.6
35	<i>L. bulgaricus</i> GM-8	18 ± 0.9	20 ± 1.4
36	<i>L. bulgaricus</i> HH-7	19 ± 0.5	16 ± 0.4
37	<i>L. bulgaricus</i> ZhZ-10	16 ± 0.4	12 ± 0.3
38	<i>L. bulgaricus</i> SS-9	11 ± 0.3	8 ± 0.2
39	<i>L. bulgaricus</i> N-7	7 ± 0.2	7 ± 0.2
40	<i>L. plantarum</i> G-24	8 ± 0.2	9 ± 0.2
41	<i>L. plantarum</i> L-15	15 ± 0.4	17 ± 0.6
42	<i>L. plantarum</i> PR-15	13 ± 0.3	10 ± 0.2
43	<i>L. plantarum</i> AR-2	8 ± 0.2	10 ± 0.2
44	<i>L. plantarum</i> BI-4	10 ± 0.2	12 ± 0.3
45	<i>L. casei</i> subsp. <i>casei</i> I-4	7 ± 0.2	9 ± 0.2
46	<i>L. casei</i> subsp. <i>casei</i> Yan-10	12 ± 0.3	15 ± 0.4
47	<i>L. casei</i> subsp. <i>casei</i> NE-12	13 ± 0.3	10 ± 0.2
48	<i>L. casei</i> subsp. <i>casei</i> EE-2	15 ± 0.4	17 ± 0.6
49	<i>L. brevis</i> GT-5	8 ± 0.2	9 ± 0.2
50	<i>L. brevis</i> Bo-2	17 ± 0.7	15 ± 0.4
51	<i>L. brevis</i> GM-26	17 ± 0.7	19 ± 0.5
52	<i>T. kefir</i> var. <i>kumis</i> 23	21 ± 1.2	18 ± 1.1
53	<i>T. kefir</i> var. <i>kumis</i> ShT-1	12 ± 0.3	10 ± 0.2
54	<i>T. kefir</i> var. <i>kumis</i> ZhSh-3	17 ± 0.6	16 ± 0.4
55	<i>T. kefir</i> var. <i>kumis</i> KM-12	11 ± 0.2	14 ± 0.3
56	<i>T. kefir</i> var. <i>kumis</i> LB-23	10 ± 0.2	8 ± 0.2

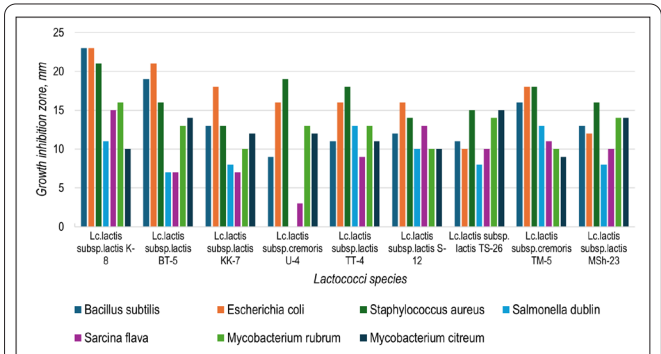


Figure 2: Antagonistic activity of *Lactococcus* subsp. *lactis* and *cremoris* species on SM.

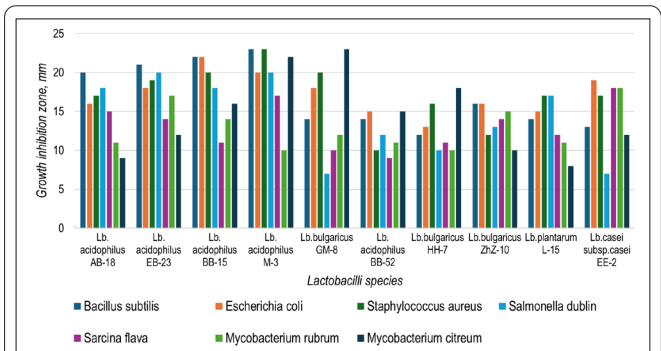


Figure 3: Antagonistic activity of *L. acidophilus*, *L. bulgaricus*, and *L. plantarum* species on SM.

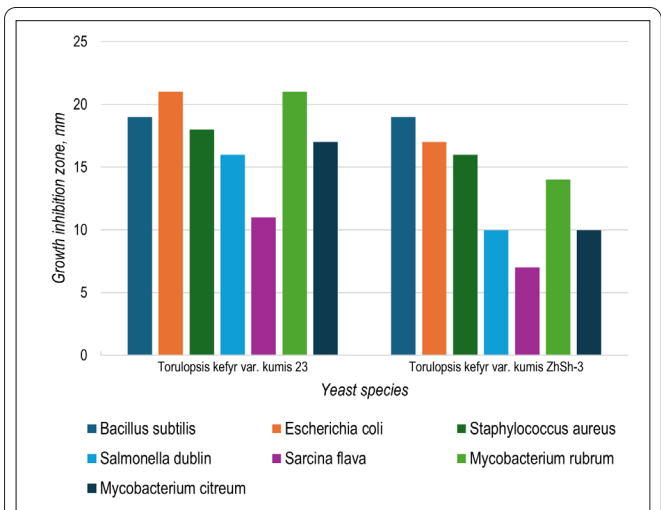


Figure 4: Antagonistic activity of yeast species *T. kefir* var. *kumis*.

When comparing the ability of acid formation of cultures with their antagonistic activity, it can be concluded that the more acid a culture forms, the more its antagonism manifests itself.

To enhance the new antagonistic properties of the cultures, two nutrient media were used in further studies of antagonistic activity: MH and synthetic MRS organic medium, most often used in production to preserve cultures of lactic acid bacteria and the preparation of starter cultures.

A more pronounced antagonistic activity is manifested in all bacteria grown on MH, compared with cultures grown on

SM. The optimal environment for the manifestation of antagonistic properties and storage in the collection is the MRS environment.

Cultivation of all strains on the MRS medium showed that their antagonistic activity was much higher than the antagonistic activity of cultures grown on SM and MH. They inhibit the growth of all used test cultures with maximum zones. An increase in the antibiotic activity of cultures of lactic acid bacteria cultivated on the MRS medium with sucrose.

Only in *L. acidophilus* EB-23 cultures, with the growth of MH, there was a slight decrease in antagonistic activity against *S. aureus*. All cultures studied by us, grown on MRS and MH, inhibit the growth of *M. rubrum* and *M. citreum* (Table 2).

MH differs from simple SM by the presence of more non-protein nitrogen, carbohydrates, and other substances necessary for the development of culture [27].

Therefore, on the MRS and MH media, Lactobacilli produces a larger amount of acid, which itself, being a growth-suppressing agent, may enhance the effect of other substances, presumably bacteriocins.

Sensitivity and resistance of selected strains, lactic acid bacteria, and yeast to antibiotics

The introduction of antibiotics into clinical practice has sharply limited the spread of many epidemic diseases. This led to several undesirable consequences, among which are the spread of antibiotic-resistant bacteria and the disruption of evolutionarily developed host microbiocenoses [28].

When selecting strains of microorganisms for fermentation, it is necessary to determine their sensitivity to antibiotics and other agents used in medicine and veterinary medicine.

All bacteria under study proved to be resistant to tetracycline. The *L. acidophilus* M-3 strain is sensitive to levomycitin

and erythromycin and shows resistance to other medicines. *L. lactis* subsp. *lactis* MSh-23 and *L. lactis* subsp. *lactis* TT-4 cultures are weakly sensitive to vancomycin, more sensitive to streptomycin, levomycitin, benzylpenicillin, and ampicillin, and resistant to other antibiotics. The *L. bulgaricus* HH-7 strain is resistant to streptomycin, levomycitin, and ampicillin and is sensitive to other medicines. *L. lactis* subsp. *cremoris* U-4 and *L. lactis* subsp. *cremoris* TM-5 strains are weakly sensitive to erythromycin, levomycitin, and benzylpenicillin, more sensitive to gentamicin, and resistant to three medicines. The *L. acidophilus* BB-15 strain is resistant to seven medicines and is sensitive to benzylpenicillin. *L. acidophilus* M-3 is inhibited by erythromycin and levomycitin; this culture is resistant to the other six medicines. The *L. lactis* subsp. *lactis* K-8 strain is resistant to all tested medicines, except vancomycin. Strains *L. bulgaricus* GM-8 and *L. acidophilus* B-52 are resistant to tetracycline and vancomycin and are sensitive to the other agents. *L. plantarum* L-15 culture is weakly sensitive to benzylpenicillin, more sensitive to erythromycin, streptomycin, and gentamicin, and resistant to other antibiotics. The strain of *T. kefyri* var. *kumis* 23 is resistant to six medicines out of eight; the rest show weak sensitivity. *T. kefyri* var. *kumis* ZhSh-3 is resistant to erythromycin, benzylpenicillin, gentamicin, and tetracycline and shows sensitivity to other medicines. All data are shown in table 3.

Analyzing the results, we can say that the studied cultures are mainly sensitive to ampicillin, levomycitin, and streptomycin and are slightly sensitive or completely insensitive to the rest of the medicines.

Such cultures as *L. lactis* subsp. *lactis* K-8, *L. acidophilus* BB-15, *L. acidophilus* M-3, and *T. kefyri* var. *kumis* 23 are resistant to almost all antibiotics. Lactobacilli are more resistant to the action of antibiotics. The data obtained by us are consistent with the literature [29].

Table 2: Antagonistic activity of lactic acid bacteria during growth on various media.

Strain	Nutrient media	Test cultures, inhibition zone in mm						
		<i>B. subtilis</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>S. dublin</i>	<i>S. flava</i>	<i>M. rubrum</i>	<i>M. citreum</i>
<i>L. lactis</i> subsp. <i>lactis</i> K-8	SM	23	23	21	11	15	16	10
	MH	23	24	21	12	18	19	13
	MRS	25	27	23	15	21	22	17
<i>L. lactis</i> subsp. <i>lactis</i> BT-5	SM	19	21	16	7	7	13	14
	MH	20	23	16	8	7	16	17
	MRS	22	22	18	11	10	20	21
<i>L. acidophilus</i> BB-15	SM	22	22	20	18	11	14	16
	MH	25	23	25	20	12	14	19
	MRS	28	32	31	24	15	19	21
<i>L. acidophilus</i> M-3	SM	23	20	23	20	17	20	22
	MH	27	23	26	23	17	23	25
	MRS	32	27	30	27	19	27	32
<i>L. acidophilus</i> EB-23	SM	21	18	19	20	14	17	12
	MH	21	20	17	22	14	17	15
	MRS	23	25	24	25	17	22	17
<i>L. bulgaricus</i> GM-8	SM	14	18	20	7	10	12	23
	MH	16	19	23	7	13	17	25
	MRS	19	24	29	14	15	23	31

Table 3: Sensitivity and resistance of the studied bacterial strains to medicines.

No.	Culture name	Erythromycin	Streptomycin	Levomyctin	Tetracycline	Ampicillin	Benzylpenicillin	Vancomycin	Gentamicin
		Growth inhibition zones (mm)							
1	<i>L. lactis</i> subsp. <i>lactis</i> K-8	-	-	-	-	-	-	22	-
2	<i>L. lactis</i> subsp. <i>lactis</i> BT-5	10	11	10	-	12	9	9	12
3	<i>L. lactis</i> subsp. <i>lactis</i> KK-7	25	-	-	13	15	-	24	15
4	<i>L. lactis</i> subsp. <i>lactis</i> MSh-23	-	11	-	-	12	12	9	-
5	<i>L. lactis</i> subsp. <i>lactis</i> TT-4	-	12	14	-	-	11	9	-
6	<i>L. lactis</i> subsp. <i>lactis</i> S-12	8	11	-	-	14	12	8	13
7	<i>L. lactis</i> subsp. <i>lactis</i> TS-26	7	10	25	15	-	10	14	-
8	<i>L. lactis</i> subsp. <i>cremoris</i> U-4	10	-	12	-	-	9	-	21
9	<i>L. lactis</i> subsp. <i>cremoris</i> TM-5	10	-	12	-	12	9	21	21
10	<i>L. acidophilus</i> AB-18	8	20	22	-	-	10	-	7
11	<i>L. acidophilus</i> EB-23	26	22	20	-	23	-	-	20
12	<i>L. acidophilus</i> BB-15	-	-	-	-	-	20	-	-
13	<i>L. acidophilus</i> M-3	19	-	21	-	-	-	-	-
14	<i>L. bulgaricus</i> GM-8	7	11	13	-	17	10	-	18
15	<i>L. acidophilus</i> B-52	22	18	27	-	20	15	-	24
16	<i>L. bulgaricus</i> HH-7	18	-	-	-	-	12	11	17
17	<i>L. bulgaricus</i> ZhZ-10	12	10	7	-	16	14	10	8
18	<i>L. plantarum</i> L-15	14	14	-	-	-	12	-	13
19	<i>L. casei</i> subsp. <i>casei</i> EE-2	12	-	6	-	-	-	7	12
20	<i>T. kefir</i> var. <i>kumis</i> 23	-	-	-	-	-	15	17	-
21	<i>T. kefir</i> var. <i>kumis</i> ZhSh-3	-	10	10	-	11	-	9	-

Conclusion

We selected and studied active strains of lactic acid bacteria and yeast from products of mixed lactic acid fermentation in various regions of Kazakhstan for starter cultures in the production of fermented dairy products. In particular, the antimicrobial properties of isolated cultures of lactic acid bacteria were studied, and the sensitivity of isolated cultures of lactic acid bacteria to antibiotics was determined.

The cultures of *L. lactis* subsp. *lactis* K-8, *L. acidophilus* BB-15, *L. acidophilus* M-3, and *T. kefir* var. *kumis* 23 exhibit resistance to nearly all antibiotics. Generally, lactobacilli demonstrated greater antibiotic resistance. Based on these findings, it is suggested that these selected cultures could be strategically utilized in the development of starter cultures.

Acknowledgements

None.

Conflict of Interest

None.

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