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SCIENTIFIC AND PRACTICAL BASICS TECHNOLOGY OF FUNCTIONAL DAIRY PRODUCTS IN MONGOLIA

DISSERTATION ABSTRACT

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The defense of dissertation will be ___ _____ 2022 at ____ according to the meeting of the Scientific Jury, organized by order RD16-824/13.July 2022 of the Rector of the Agricultural University-Plovdiv. The members were at the meeting:

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The research was carried out in the research laboratory "Food research, innovative open center" of the Industrial Technology School of the Mongolian University of Science and Technology, research laboratory (Japan), Moscow State Research Institute of Genetics and Selection of Industrial Microorganisms, Research laboratory of milk and dairy products of Konkuk University (South Korea) and in the laboratory of milk and dairy products of the Moscow State University of Applied Biotechnology, and in the research laboratory of Agricultural University-Plovdiv.

The dissertation consists of the following main chapters: Introduction, Analytical review of information sources, organizations, objects and Methods of research, Results of own research, Practical implementation of research results, Conclusion and List of references and Appendixes. The main content of the study is set out on 288 pages of typewritten text, contains 73 tables, 25 figures and graphs. The list includes 257 literature sources.

The dissertation is located in the library of the Agricultural University, at the address: 4000, Bulgaria, Plovdiv, blvd. Mendeleev 12, and can be introduced to the abstract and other materials on the site https://www.au-plovdiv.bg/

Dissertation abstract was sent out on July 29, 2022.

GENERTAL DESCRIPTION OF THE DISSERTATION

Relevance of the research topic. In recent years functional purpose dairy production has challenge in Mongolia.

One of the directions for creating dietary and medicinal dairy products is to enrich them with protective factors, in particular microorganisms that are part of the normal microflora human intestines.

Currently widely used probiotic dairy products, as probiotics make up basis of normal microbiocenosis and takes direct participation in the process of digestion.

Nowadays, in Mongolia, special attention is paid to the problem production for children's and dietary dairy products and cheese in Mongolia and the production of probiotic fermented milk products the main raw material is cow’s milk. However, there has been increased interest in use of goat and sheep milk as a raw material for industrial production of dairy products, especially in Mongolia goat breeding is developing intensively and goat milk is a valuable diet product. Goat and sheep milk as a raw material for industrial production dairy products in Mongolia are not sufficiently studied and not developed science-based technologies for products based on goat and sheep milk.

In this regard, the development of science-based technologies production of products based on goat and sheep milk, containing representatives of beneficial intestinal microflora is an urgent problem that has an important medical and national economic importance.

At the same time, the isolation and study of probiotic lactic acid bacteria strains from traditional dairy products in the use of production play an important role in functional foods.

The purpose of the work and the objectives of the research. The purpose of the dissertation is to give a scientific rationale and develop a technology for functional dairy products using new types of starters culturs, prepared the strains of lactic acid bacteria are isolated from Mongolian tradional dairy products.

In accordance objective, the following research tasks are defined:

1. Studying the chemical composition of goat, sheep and cow's milk of pasture-raised local animals of Mongolia;
2. Isolating strains of lactic acid bacteria from Mongolian traditional dairy products;
3. Identifying microorganisms, isolated from traditional dairy products;
4. Studying the probiotic properties of lactic acid bacteria strains;
5. Making a science-based selection for the type of starter cultures to create functional products;

6. Substantiating the methodology of the obtained bacterial starter cultures for fermented dairy products;
7. Justifying the prospects of technological regimes of fermented milk and cheese products made by goat, sheep and cow's milk;
8. Developing technologies for probiotic, synbiotic fermented milk and cheese using goat, sheep and cow's milk;
9. Determining the nutritional, biological and energetic value of new types of functional products;
10. Conducting clinical trials of dairy products with probiotic properties;
11. Conducting industrial testing and implementation based on the research results of industrial technology.

Scientific novelty. A comprehensive assessment of the quality and technological properties of the milk of the Mongolian breed goats and sheep was carried out using modern methods of analysis. The selection has been made identification and study of the probiotic properties of strains, isolated from national dairy products. It has been established that the composition of the microflora of national fermented milk products tarag, airag, khoormog, byaslag which are rich of microorganisms and yeasts.

The composition of microorganisms isolated from national fermented products for sourdough production: *Lactobacillus L. paracasei subsp. paracasei, Lactobacillus paracasei subsp. tolerans, Lactobacillus delbrueckii subsp. lactis, Lactobacillus fermentum, Streptococcus salivarius subsp. thermophilus, Lactobacillus. helveticus, Lactobacillus fermentum.*

Optimal conditions for milk fermentation have been selected by new starter cultures isolated from Mongolian traditional dairy products. The main technological parameters of production and technologies have developed on the scientific basics for fermented products from

Theoretical and practical significance of the work. Thesis is a scientific qualification work with the following scientifically based technological solutions, implementation which makes a significant contribution to the development dairy industry. A technology has been developed for obtaining a starter containing in its composition of a culture of lactic acid bacteria with probiotic properties isolated from traditional dairy products.

New groups of probiotics, synbiotic and protein products, characterized by increased nutritional and biological value and manufactured according to resource-saving technologies.

The novelty of the technical solutions underlying the developed technologies, confirmed by copyright certificates and patents for invention. Research materials have been published in three monographs.

Theoretically substantiated and practically implemented innovative technology of a number of pro-prebiotic and protein products in Mongolia and abroad.

Publications. Was published in 36 scientific publications on the dissertation work, 10 of them in refereed scientific journals.

Journal of Food Science & Nutrition, Journal of Food Science and Technology Research, Journal of Functional Foods, Journal of International Immunopharmacology, Journal of Human Cell, Journal of Animal Science, Journal of Foods and Raw Materials, Journal of International Food Research, Korean Journal of Dairy Science and Technology, Mongolian Journal of Chemistry), Journals: Food industry, Dairy industry, Storage and Processing of Agricultural Raw Materials, Technique and Technology Food Production, 8 articles in journals recommended by the Higher Attestation Commission of Russia for publication of the main materials of my dissertation research and conference presentations in the proceedings, R&D reports, as well as Mongolian patents for results of my research findings.

Structure and volume of dissertation work. The dissertation consists of 8 chapters, including introduction, analytical literature review, methodological part, results of own research. The main content of the dissertation is presented on 288 pages.

RESEARCH RESULTS AND DISCUSSION

Analytical review consists of sections in which analysis of information from sources of foreign and domestic literature on the problems that are related to the study. The medical and biological justification for the creation of functional food products were determined during the study.

Moreover, the characteristics of dairy raw materials were given: goat's milk, sheep's and cow's milk, as an object for children's food, also for probiotic and protein products from these types of milk. From the review material it follows that the current level of the dairy industry country and its resource base state requires the creation and implementation technologies for functional purpose products also were considered features of traditional dairy products. The prospects for enzyme biotechnology development of dairy products and preparations with probiotic properties are shown and are determined their importance for the population's health improvement at the present stage.

In the chapter the composition of beneficial microflora was substantiated which used for the production of fermented dairy products and preparations of probiotic action.

Data on modern achievements in genetics and genetic engineering research methods were given it was considered to explore the issues of obtaining single- and multi-species associations of microorganisms, as well as the trend and prospects for probiotics production.

Organization of work, objects and methods of research

The object of research was goat, sheep and cow milk, collected in the private animal farms in Mongolia. Besides theses types of milk some Mongolian traditional fermented milk products as tarag (identical to yogurt), khoormog (fermented milk product from camel milk), airag (fermented milk drink made from mare's milk), byaslag (a type of cheese) and other products were also object of the study. Samples of traditional Mongolian dairy products were selected from different regions of the country. Strains from traditional Mongolian dairy products were used to create starter cultures. The general scheme of research is presented in figure 1.

For the study was used standard methods of microbiological research and genetic methods, in particular 16S-ribosomal DNA. When organizing and conducting research was used generally accepted standard complex, including physicochemical, microbiological, biochemical, rheological, and mathematical methods of statistical processing of research results and construction of mathematical models

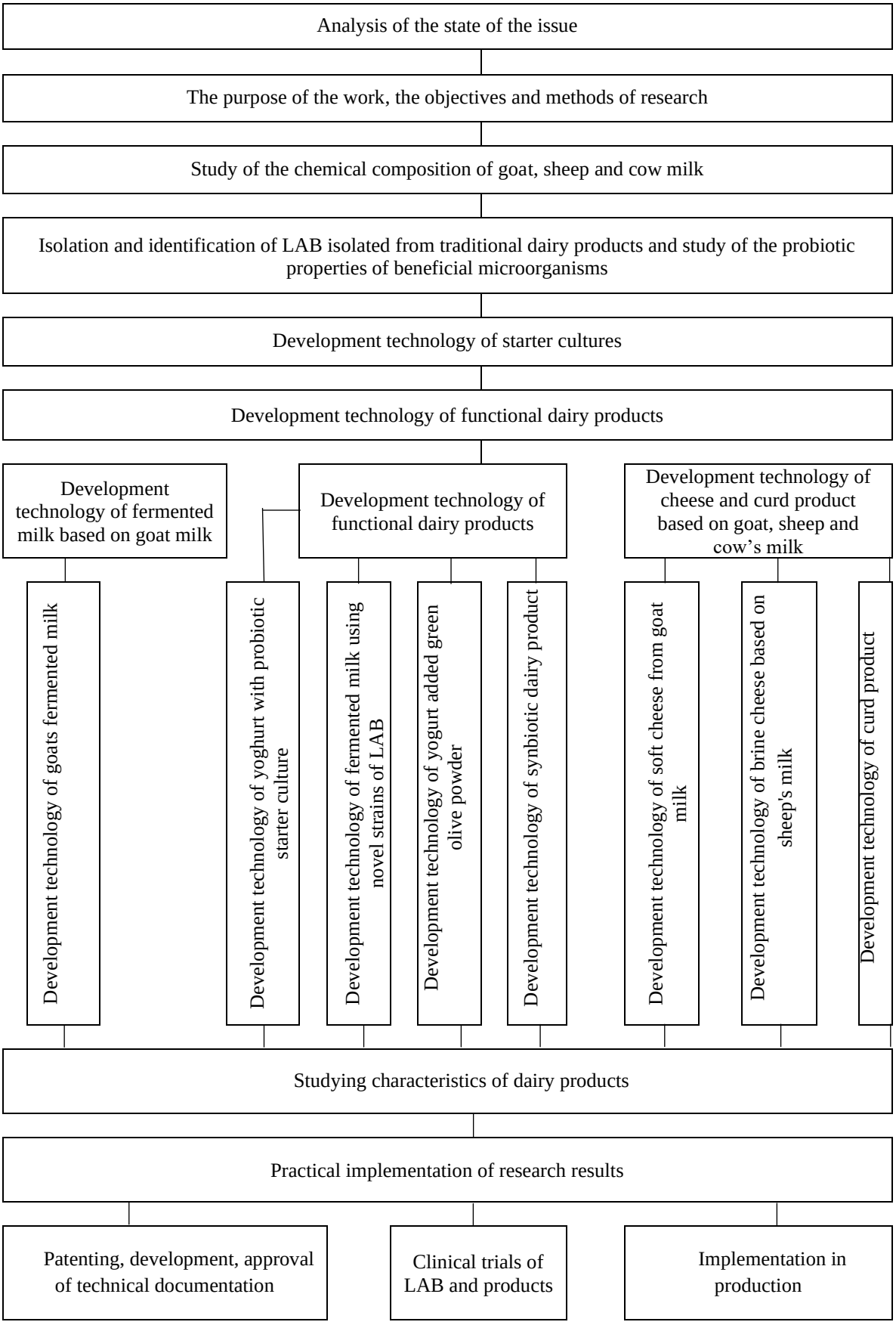

Figure 1. The general methodology of research

I. Study of the physico-chemical composition and safety of milk some farm animals of Mongolian breeds

In Mongolia, unlike other countries of the world, traditionally dairy products are prepared from milk of five animal species (cow, mare, goat, sheep, camel), which is one of distinctive features. However, there is industrially processied mostly cow's milk in our country, but milk from other animal species used to prepare traditional dairy products in a small amount in individual farms. In Mongolia, according to statistics in 2020, there were about 67.0 million heads of livestock, of which 27.2 million are goats and 30.0 million are sheep, as a result, and the potential resources of goat and sheep milk have increased. For example, in 2020 the industry could be processed approximately 259.6 million liters of goat's milk, 165.6 million liters of sheep's milk [www.1212].

The study of the physico-chemical composition of milk pasture-raised animals of Mongolia

To implement measures to organize the industrial processing of goat's and sheep's milk of Mongolian breed the physicochemical characteristics of the goat's and sheep's milk including amino acids, fractional composition, minerals and vitamin composition of goat, sheep and cow's milk were studied.

As a result of research, the milk of pasture-raised local animals of Mongolia is characterized by a high dry matter content and rich in fat and protein.

As you know, the biological value of milk is determined not only absolute content of proteins, but also the essential amino acid content in its composition. In this regard, we conducted a study of the amino acid content of proteins in combined milk samples. Research results were shown in table 1.

Table 1. Amino acid content of goat, sheep and cow milk of Mongolian breed

Amino acid, %	Milk		
	Goat	Sheep	Cow
Valine	6.34	6.39	5.89
Isoleucine	5.19	5.41	5.27
Leucine	9.38	9.69	9.73
Lysine	8.11	8.55	8.16
Methionine	1.73	2.38	2.08
Tryptophan	4.44	4.15	4.04
Tyrosine+Threonine	3.32	3.47	3.54
Phenylalanine	4.61	4.81	4.41
Alanine	3.93	2.77	4.82
Arginine	3.13	3.63	3.12
Aspartic acid	8.05	8.10	8.21
Histidine	1.74	1.80	1.79
Glycine	2.03	2.04	2.20
Glutamic acid	22.56	21.50	22.40
Proline	10.00	9.85	8.82
Serine	4.32	4.06	4.46
Cysteine	2.17	2.03	1.80
Total	101.05±0.72	100.63±0.58	100.64±0.50

The data presented in the table 1 are shown, that the goat, sheep and cow milk are characterized by high amino acid content such as glutamic acid, aspartic acid, leucine, lysine leitsin, lizin and proline.

Content essential amino acids in the total protein of the goat, sheep, and cow milk of the Mongolian breed with values of 43.64%, 45.41%, and 44.49%, respectively. According to these data as goat, sheep and cow milk have similar food value.

It was the reason for studying whey protein's fractional composition of goat milk. The data of study was as shown in table 2.

As shown in table 2 goat's milk is different from cow's milk on serum albumin, β-Lactoglobulin, α-Lactoalbumin fractions. According to the amount of α -lactalbumin fraction, protein in goat's milk is significantly higher than protein in cow's milk.

Table 2. Fractional composition of whey proteins of goat milk

Milk	Fractional content, %			
	Serum albumin	β-Lactoglobulin	α-Lactoalbumin	Immunoglobulins
Goat	8,95±1,50	20,87±1,90	58,3±1,90	11,80±0,80
Cow	10,20±0,88	45,5±1,59	18,11±2,09	12,62±1,36

As can be seen from table 3, according to the calcium content of goat milk higher than in cow's milk, but when compared with sheep's milk.

Table 3. Mineral composition of Mongolian pastorals animal's milk

Micro and macro elements	Mineral composition in milk, mg% of ash		
	Goat	Sheep	Cow
Calcium	22,88±1.50	28,56±2.27	18,83±1.46
Sodium	4,15±2.42	7,03±2.56	4,76±1.95
Potassium	16,90±0.06	13,0 ±0.41	22,4±0.59
Magnesium	1,53±1.17	2,16±1.60	1,64±3.46
Phosphorus	17,48±0.44	15,12±0.51	18,40±0.74
Zinc	0,05±0.86	0,08±2.20	0,04±0.41

In the table 3, calcium in goat milk is more than in cow milk, but it is much lower than in sheep milk. Calcium, sodium and magnesiumcontents in sheep milk are higher than in goat and cow milk. According to the results that the sheep milk contains approximately twice more sodium, than goat and cow milk but sodium is approximately similar in contents.

Compared to the other two types of milk, cow's milk contains slightly more potassium.

As shown at table 4 quantitative content of vitamin B1 and B2 in sheep's milk are higher than the goat's and cow's milk. Content of vitamin A in cow's milk is lower than in goat's and sheep's milk.

Table 4. Vitamin composition goat, sheep and cow's milk

Vitamins, μg/100g	Milk		
	Goat	Sheep	Cow
Thiamin ($B_{1)}$	38,96	74,85	30,08
Riboflavin (B_2	156,12	417,66	180,37

Retinol (A)	381	465	146
Dl-α-tocopherol (E)	1717	1870	1855

The study of the safety analyses of goat and sheep milk from different regions of Mongolia

Toxic elements and radionuclide were identified and assessed safety of goat and sheep's milk.

Table 5. Heavy metals concentration and pesticides in goat and sheep's milk in Mongolia.

Hazardous elements	Content, mg/kg of milk		Permissible level, mg/kg, less than
	Goat	Sheep	
Lead	0.057	0.048	0.1
Arsenic	0.0024	0.012	0.05
Cadmium	0.019	0.010	0.03
Mercury	0.004	0.003	0.005
GCTG-isomers	0.008	0.008	0.05
DDT and its metabolites	0.005	0.005	0.05

The results of data analysis showed that the content of the studied elements in all milk samples did not exceed the maximum permissible level of concentration established by technical requirements.

Table 6. Radionuclide content of goat and sheep's milk in Mongolia

Hazardous elements	Content, Bk/kg (L)		Permissible level, Bk/kg (L)
	Goat	Sheep	
Strontium-90	0.48	0.33	25
Cesium-137	1.11	3.19	100

After determining the content of radionuclides (table 6) in goat and sheep's milk was shown the following results: strontium-90: 0,48, cesium-137: 1,11 Bk/kg (L) in goat's milk and in sheep's milk 0,33; 3,19 Bk/kg (L), respectively. It should be noted that radionuclides are higher contained in sheep's milk that in goat's milk. Thus, analyzing the data obtained, it is necessary note that these types of raw materials are safe for the nutrition products.

Research results confirm the great value of goat and sheep milk of Mongolian breed animals, safety of use it as a raw material for food production, as well as the need for further detailed studies to substantiation of parameters of industrial technology of products preventive focus.

Evaluation of of goat and sheep's milk quality was carried out in accordance with the requirements for cow's milk.

II. Isolation and identification of strains of lactic acid bacteria and the study of their probiotic properties.

Mongolian food culture, especially concerning dairy products, is different from Western cultures. In Mongolia, in contrast to other countries of the world, dairy products are traditionally prepared from the milk five kinds of animals (cows, mares, goats, sheep and camels).

However, it is industrially processed milk and dairy products mainly of cow's milk, while the milk of other animal species is used for the preparation of traditional dairy products in a small volume in individual farms.

In other countries to produce fermented milk products use starter cultures consisting of specific strains bacteria, then for the manufacture of Mongolian traditional dairy products they use the indigenous natural starter culturs (hurunge). The use of starter cultures has long been a custom of Mongolians. The indigenous starter culture that is used to make traditional fermented dairy products.

At the same time, the microflora in home-use, the indigenous starter cultures, passed down from generation to generation, is practically not studied. There are no self-developed starter cultures for fermented milk products, including functional starter cultures. In this regard, the isolation and identification of strains inside Mongolian traditional dairy products have scientific and practical interest.

Isolation and Identification of strains of LAB

The aim of this chapter was to study and evaluate the probiotic potential of lactic acid bacteria (LAB) isolated from traditional Mongolian dairy products. The object ofour study was fermented milk products, produced by the traditional method. A total of 88 samples of traditional Mongolian fermented milk (tarag, khoormog, airag, aaruul, byaslag and eezgii) were collected from different regions of Mongolia.

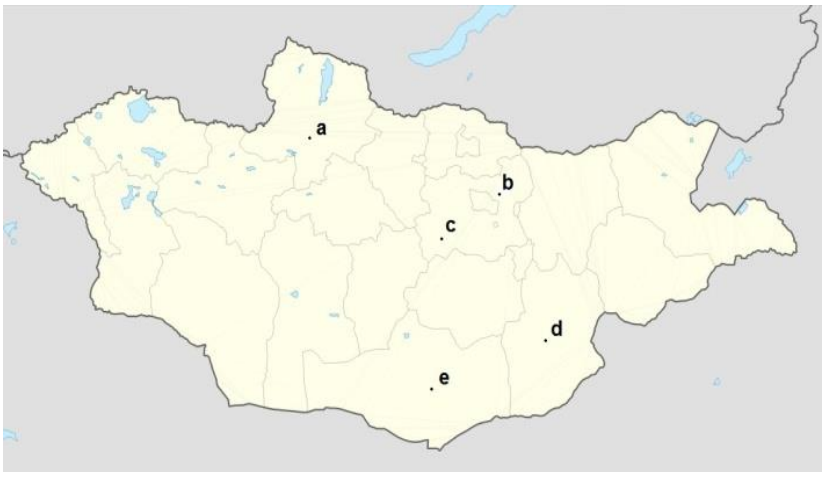

Figure 2. Regions where were collected samples of fermented milk on the map Mongolia:

a – city Ulaanbaatar, b – Altanbulag, Tov aimag, c – Muren, Khubsugul aimag, e – Sainshand, Dornogobi aimag, e – Dalanzadgad, Umnugobi aimag.

The analysis of the products showed that the following microorganisms are contained in the traditional fermented milk products: *Streptococcus thermophilus, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus helveticus, Lactobacillus fermentum, Lactococcus lactisssp.lactis, Lactobacillus pentosus, Weissella confuse, Lactobacillus kefiri, Lactobacillus plantarum, Lactobacillus. paracaseissp. tolerans, Pediococcus parvulus, Lactobacillus. paracasei ssp. paracasei, Leuconostoccitreum, mesenteroides, Weissellaviridescens, Lactobacillus sakei, Weissella confuse, Lactobacillus pentosus, Lactobacillus buchnri, Leuconostoccitreum, Leuconostocgarlicum, Enterococcus durans, Enterococcus faecium, Leuconostocmesenteroides, Bacillus lechiniformis and Brevibacillusinvocatus and* yeast.

Identification of lactic acid bacteria

For preliminary identification, all strains from MRS and GYP were Gram-stained and tested for catalasing activity. From samples of traditional dairy products isolated 587 isolates, all of which were Gram-positive and catalase-negative. Strains that were Gram-positive and catalase-negative, classified as lactic acid bacteria.

Strains isolated from the traditional dairy products identified based on the 500 base pair (bp) sequences from the 5′-end of 16S ribosomal DNA (16S rD-NA).

Table 7. The number of strains of lactic acid bacteria isolated from traditional dairy products

Fermented milk	Numbers of strains of LAB isolated from fermented milk	
	Numbers	Rate, %
Tarag and khoormog	462	78,7
Airag	68	11,6
Aaruul	41	7,0
Byaslag	16	2,7

The most strains of lactic acid bacteria were isolated from tarag and khoormog 78.7% (462 strains), from airag 11.6 % (68 strains), from aaruul – 7.0% (41 strains), from byaslag – 2.7% (16 strains).

462 strains of LAB were isolated from tarag and khoormog, and classified into 17 species by16S rDNA sequence. *L. delbrueckii ssp. bulgaricus, L. helveticus, L. fermentum, str. salivarius subsp. thermophilus* were the majority of detected species, and are assumed to be predominant in tarag and khoormog. At the same time, *L. delbrueckii ssp. bulgaricus* was dominated in tarag 36.2%. As you know, beneficial microflora in typical yogurt is *L. delbrueckii ssp. bulgaricus and S. salivarius subsp. thermophilus,* which are produced using a similar technology. Therefore, tarag can be considered as the same type as yogurt fermented milk product, but only with the inclusion of uncharacteristic for yogurt microorganisms, which is associated with national characteristics obtaining dairy products in Mongolia

Detected 68 strains in airag samples that were classified into 12 species by 16S r DNA. The predominant LAB species from airag was *L. helveticus. L. delbrueckii ssp. lactis, L. fermentum u Weissella* (W.) viridescens, which differs slightly from reports from [Uchida et al. 2007 and Watanabe et al. 2008].

From aaruul isolated 41 strains which classified into 10 species. The most frequently detected of which were *L. fermentum* (26,8 %), *L. helveticus, L. buchneri u W. confuse.* From byaslag isolated 16 strains were detected and identified as seven different species, the most frequent of which were *L. delbrueckii ssp. Lactis and Lactococcus lactis ssp. Lactis.*

As is known as a beneficial microflora in typical yoghurts is *L. delbrueckii ssp. bulgaricus* and S. *salivarius subsp. thermophilus,* which are produced using a similar technology. Therefore, tarag can be considered as the same type of fermented milk product as yogurt, but only with the inclusion of microorganisms uncharacteristic of yogurt, which is related to the national characteristics of the producing of dairy products in Mongolia.

67 strains have been isolated from airag and they are classified into 12 types. The predominant species of LAB in airag were *L. helveticus, L. delbrueckii ssp. lactis, L. fermentum and Weissella viridescens.* These data differ slightly from reports by [Uchida *et al.*,2007 and Watanabe *et al.*,2008].

41 strains were isolated from aaruul, which were classified into 10 species, the most frequently detected species was *L. fermentum* (26.8%). 15 strains were isolated from byaslag.

The dominant representatives of LAB in aaruul were *L. fermentum, L. helveticus, L. buchneri* and *W. confuse*, while from byaslag dominated by *L. delbrueckii ssp. lactis and L. lactis spp. lactis.*

It was found that aaruul contains homofermentative lactic acid bacteria L. helveticus and L. delbrueckii ssp. lactis and also heterofermentative lactic acid bacteria L. fermentum, L. buchnerii and W. confuse, while L. delbrueckii ssp. lactis and L. lactis spp. lactis.

There is a possibility that heterofermentative LAB in aaruul could be introduced from the external environment, since according to the traditional processing technology aaruul made by boiling taraga and then drying in the sun for approximately an hour. We suggest that these processes might kill LAB from tarag. This supports the hypothesis that almost all LAB in aaruul were of environmental origin.

Most of the isolated strains are assigned to the following taxonomic groups: *Lactobacillus. delbrueckii ssp. bulgaricus, Lactobacillus helveticus, Lactobacillus fermentum, Streptococcus thermophilus.*

The presented results of strains identification of microorganisms from a product with the same name, but obtained on the basis of milk from different farm animals, also indicate the diverse composition of the microflora present in them. This can be explained by the fact that the culture and production of Mongolian dairy products is different, and therefore they may include potentially unusual microorganisms.

Research results allow us to conclude that the dominant types of lactic acid bacteria in tarag and hoormog are *L. delbrueckii ssp. bulgaricus, L. helveticus, L. fermentum and S. thermophilus,* which were the main among the discovered species.

Screening isolated strains for probiotic LAB

Survival of bacteria in an artificial digestive juice was analyzed by subjecting isolates to a bile acid tolerance test of growth in GYP broth with 0.2% oxgall. Of the 587 isolates, 148 could grow in nutrient broth with 0.2% oxgall, and were assumed to be tolerant to bile acid. These strains of LAB were tested for gastric acid tolerance. We assumed that LAB strain

detected at over 7 log colony forming unit (CFU)/ mL after exposure to pH 3.0 with 0.04% pepsin for 3 h could tolerate gastric acid. Of the 148-bile acid-tolerant strains, 114 were also viable in low pH with 0.04% pepsin. Homofermentative LAB is more typically used for making yoghurt than heterofermentative LAB for the quality of it. Then the LAB strains have been confirmed to produce gases from glucose. Of the 114 strains expected to tolerate digestive secretions, 42 of 114 strains showed homofermentation, which suggests utility in yoghurt production. Finally, these 42 strains were tested for adhesion to Caco-2 cells, using CFU/mL greater than 5.0×10^3 as the threshold for adhesion. Ten strains adhered to Caco-2 cells by this criterion, with colony numbers ranging from $7.0x10^3$ to $7.5x10^4$ CFU/ mL, and *L. plantarum* showing the highest values.

The most important characteristic feature of probiotic bacteria refers to their adhesive ability. The adhesion threshold was considered that the number of attached cells to the epithelium should be $5.0x10^4$ CFU/ml. 42 strains showing homofermentative properties were tested for adhesion to Caco-2 cells. It was found that 10 strains with the number of colonies from 7.0x103 to $7.5x10^4$ CFU/ml and strain *L. plantarum 05DTS23* had the highest values adhesion to Caco-2 cells.

Table 8. The screening of all lactic acid bacteria isolated from Mongolian dairy products for probiotic properties

LAB species	Detected number	Viability in bile	Tolerance in low pH	Gas procuct (-)	Adheasion on Caco-2
Lactobacillus delbrueckii ssp. bulgaricus	157	10	2	4	0
Lactobacillus helveticus	118	0			
Lactobacillus fermentum	93	65	49	0	
Streptococcus thermophilus	58	10	0		
Enterococcus durans	19	0			
Weissella confuse	16	16	16	0	
Lactococcus lactis ssp. lactis	20	2	2	2	0
Lactobacillus buchneri	7	1	1	0	
Lactobacillus kefiri	10	0			
Lactobacillus plantarum	11	11	11	11	5
Lactobacillus pentosus	13	9	9	9	0
Lactobacillus delbrueckii ssp. lactis	22	2	2	2	1
Pediococcus parvulus	5	5	5	5	0
Enterococcus faecium	9	0			
Weissella viridescens	6	6	6	0	
Lactobacillus paracasei ssp. torelans	6	5	5	5	2
Lactobacillus paracasei ssp. paracasei	4	4	4	4	2
Lactobacillus sakei	4	2	2	2	0
Leuconostoc mesenteroides	2	0			
Leuconostoc citreum	6	0			
Leuconostoc garlicum	1	0			
	587	148	114	44	10

Of the 587 strains studied, 148 were tolerant to bile acids, 114 were tolerant to gastric juice, and 10 were able to adhere to Caco-2 human intestinal cells. At the same time, the best results for all studied indicators were found in strains of *Lactobacillus plantarum, Lactobacillus paracasei ssp. paracasei, Lactobacillus paracasei ssp. tolerans* isolated from different samples of traditional dairy products.

Таблица 9. The profile of lactic acid bacteria screened for probiotics and utilized in yoghurt production

Strains	Bile acid tolerance, %	Survival at low pH	Adhesion on Caco-2 cells	Products	Milk
L. plantarum	97.0	8.1	75.0	airag	Mare's
L. plantarum	88.9	8.2	7.0	tarag	Camel's
L. paracasei ssp. paracasei	87.9	8.0	11.0	tarag	Camel's
L. paracasei ssp. paracasei	92.6	8.0	12.0	tarag	Camel's
L. paracasei ssp. tolerans	87.4	7.3	18.0	tarag	Camel's
L. plantarum	85.4	7.7	13.0	tarag	Camel's
L. paracasei ssp. paracasei	85.6	7.0	16.0	tarag	Camel's
L. plantarum	97.0	8.7	13.0	aaruul	Cow's
L. delbrueckii ssp. lactis	83.0	8.5	27.0	tarag	Cow's
L. plantarum	92.6	8.0	8.0	aaruul	Cow's

* †The percent of viable colonies in GYP broth including 0.2% oxygall compared to the control. **The viable colonies of LAB after a treatment in 0.04% pepcin at pH 3.0 for 3 hours
***The viable colonies LAB adhered on Caco-2 cells after incubation.

The profiles of carbohydrate utilization on LAB strains selected for probiotics

It is known that microorganisms differ from each other in biochemical properties - the ability to metabolize nutrients, antibiotic substances, various oxygen-containing organic compounds such as carbohydrates, sugars, alcohols and organic acids, to synthesize enzymes, proteins, amino acids and vitamins. The study of the physiological and biochemical properties of bacteria allows it is possible to separate them using special nutrient media, to identify their species and to separate them by strains. This is true for pure cultures, lactic acid and other types of bacteria used in the food industry.

The isolated strains with high adhesive capacity were analyzed for their ability to ferment carbohydrates. The data obtained are given in table 10.

Table 10. The profiles of carbohydrate utilization on lactic acid bacteria strains selected for probiotics

No. Species	05DTS23	06TSD8	06TSD19	06TSD22	06TSD39	06TSD40	06TSD43	06LH2	06DTS3	06LH9
	1	1	2	2	2	1	2	1	3	1
D arabinose	-	-	-	-	-	-	-	-	-	-
L arabinose	-	-	-	-	-	-	-	-	-	-
D ribose	+	+	+	+	+	+	+	+	-	+
D xylose	-	-	-	-	-	-	-	-	-	-
L xylose	-	-	-	-	-	-	-	-	-	-
D galactose	+	+	+	+	+	+	+	+	-	+
D glucose	+	+	+	+	+	+	+	+	+	+
D fructose	+	+	+	+	+	+	+	+	+	+
D mannose	+	+	+	+	+	+	+	+	+	+
L rhamnose	-	-	-	-	-	-	-	-	-	-
D mannitol	+	+	+	+	+	+	+	+	-	+
D sorbitol	+	+	+	+	+	+	+	+	-	+
Amygdalin	+	+	+	+	+	+	+	+	-	+
Esculin	+	+	+	+	+	+	+	+	-	+
Salicin	+	+	+	+	+	+	+	+	-	+
D celibiose	+	+	+	+	+	+	+	+	-	+
D maltose	+	+	+	+	+	+	+	+	-	+
D lactose	+	+	+	+	+	+	+	+	+	+
D melibiose	+	+	-	-	-	+	-	+	-	+
D sucrose	+	+	+	+	+	+	+	+	-	+
D trehalose	+	+	+	+	+	+	+	+	+	+
D melezitose	+	+	+	+	+	-	+	-	-	+
D raffinose	-	-	-	-	-	+	-	+	-	+
Gluconate	+	+	+	+	+	+	+	+	-	+
Starch	-	-	-	-	-	-	-	-	-	-

1 – L. plantarum; 2 – L. paracasei ssp. paracasei; 3 – L. delbrueckii ssp. lactis;
+ positive; -, negative.

The identification results of the studied strains on lactic bacteria, with the exception of the 06TSD39 strain, were consistent with the results 16S-ribosomal DNA.

Differentiation of LAB strains selected as probiotics

For determining the possibility of using newly isolated strains of lactic acid bacteria in the technology of dairy products, all their characteristics should be carefully checked. It was interesting to determine the homology between strains of lactic acid bacteria assigned to the same species, isolated from the same products and the same place of origin.

RAPD-PCR analysis was carried out to determine the homology between LAB strains isolated from the same products, from the same place and origin, in particular between *L. plantarum* 06TSD8 -*L. plantarum* 06TSD40, *L. plantarum* 06LH2 - *L. plantarum* 06LH9, *L. paracasei spp. paracasei 06TSD19* - *L. paracasei spp. paracasei 06TSD22* - *L. paracasei spp. paracasei* 06TSD43. Based on this method, strains 06TSD8-06TSD40, 06LH2-06LH9 and 06TSD19-06TSD22-06TSD43 were isolated.

Results analysis after using this method allows us to conclude that strains 06TSD8 – 06TSD40, 06LH2– 06LH9 and 06TSD19 – 06TSD22 – 06TSD43 can represent individual isolates (Fig. 3).

The study results showed that strains 06TSD8 and 06TSD40 isolated from khoormog made from camel milk from the Sainshand in Dornogovi aimag (region), and strains 06LH2 and 06LH9 isolated from tarag made from cow milk from Altanbulag in Tov aimag, belong to *L. plantarum.* Strains 06TSD19, 06TSD22 and 06TSD43 isolated from khoormog made from camel milk from Sainshand in Dornogovi aimag (region) belong to *L. paracasei ssp. paracasei.*

The main and useful microflora in typical yoghurts is *L. delbrueckiissp. bulgaricus and S. thermophilus.* Tarag producing technology is identical to that of yogurt production. Based on the results of the conducted studies, it can be concluded that the dominant species of lactic acid bacteria in tarag are *L. delbrueckiissp. bulgaricus, L. helveticus, L. fermentum, and S. thermophilus.* Therefore, tarag and yogurt can be considered the same type of dairy products. Since the culture of dairy products in Mongolia is different from that in other countries, they may include microorganisms that are not characteristic of yogurt.

As a result of the research, it was revealed that the dominant species of lactic acid bacteria in airag were *L. helveticus*, *L. delbrueckii*ssp. *lactis*, *L. fermentum*. We found that homofermentative lactic acid bacteria *L. helveticus*и *L. delbrueckii*ssp. *lactis,* as well as heterofermentative lactic acid bacteria *L. fermentum*, *L. buchneriiи W. confuse*. in aaruul. *L. delbrueckii* ssp. *lactis* and *L. lactis* spp. *lactis*. were dominated in byaslag.

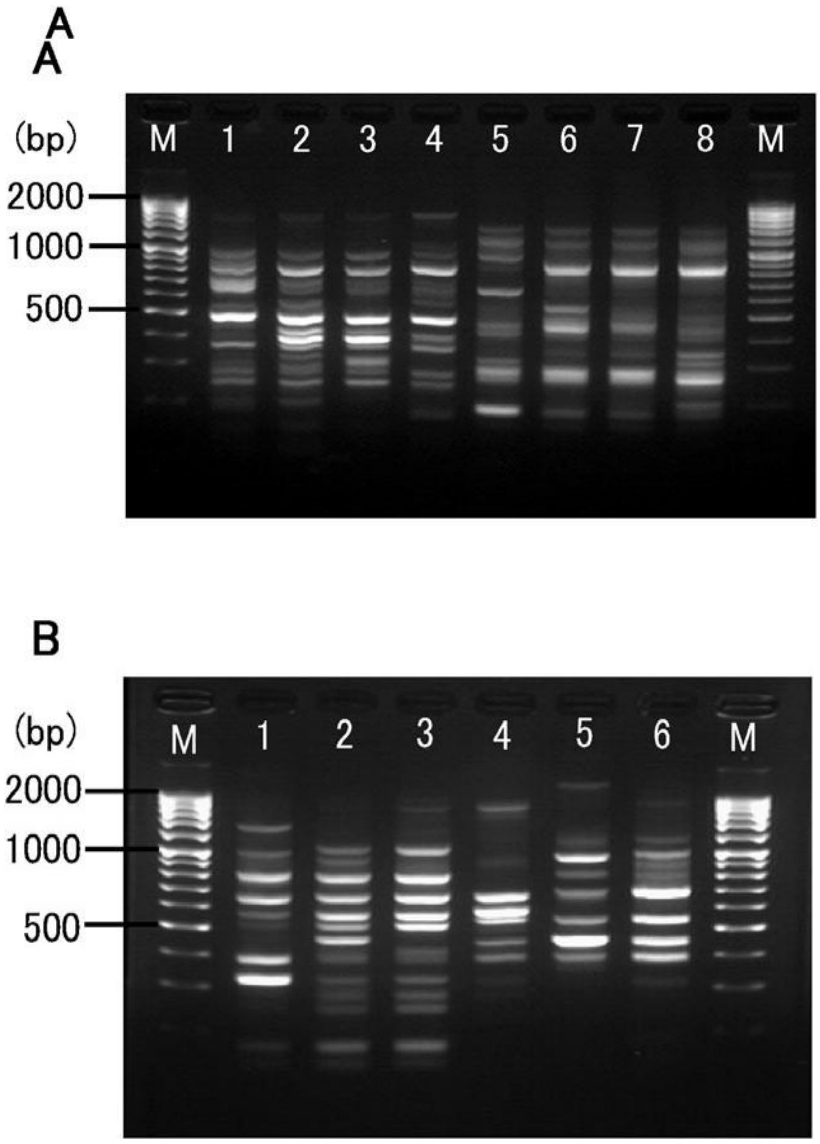

Figure 3. Random amplified polymorphic – polymerase chain reaction profiles obtained from seven lactic acid bacteria (LAB) isolates. Profiles of seven isolates of lactic acid bacteria during PCR with random amplification: a - lanes 1, 2, 5 and 6 - primer p7 was used, lanes 3, 4, 7 and 8 - primer p11 was used, lane M - DNA marker, lanes 1 and 3 – 06TSD8, lanes 2 and 4 – 06TSD40, lanes 5 and 7 – 06LH2, lanes 6 and 8 – 06LH9; (b) lanes 1, 2, and 3 - primer AT41 used; lanes 4, 5, and 6 - primer BT05 used; lane M, DNA size marker; lanes 1 and 4 - 06TSD19; lanes 2 and 5 - 06TSD22; lanes 3 and 6 - 06TSD43.

According to the aaruul production process, it is made by boiling tarag for approximately an hour, followed by sun-drying.

Eezgii is made by a complete evaporation of highly fermented milk, followed by crushing and sun-drying. Since we did not detect LAB strains in eezgii, we propose that

evaporation and sun-drying processes had an impact on the moisture content of eezgii, which reduces the total survival strains of LAB.

It has been established that 6 out of 10 strains of lactic acid bacteria isolated from hoormog have probiotic activity. Of the 54 lactic acid bacteria identified as *L. plantarum or L. paracasei ssp.,* 10 were isolated from khoormog made from camel milk.

We found that 6/10 probiotic LAB strains were isolated from camel milk tarag from the Dornogobi aimag. Of 54 LABs from tarag made with camel milk, 11 strains were identified as *L. plantarum or L. paracasei ssp.* Although these LAB species might be indigenous in Dornogobi aimag, they appeared to be more frequent in tarag made with camel milk than that with other animal milks.

Thus, the isolated and identified strains of lactic acid bacteria with probiotic properties can be recommended for inclusion in the collection of Mongolian microorganisms and for the creation of starter cultures used in the fermented milk products producing.

New strains of microorganisms isolated from Mongolian traditional dairy products will serve as the basis for the creation of a national collection of microorganisms in Mongolia and their use in the composition of starter cultures for the industrial production of fermented milk, including those with functional properties.

III. **Scientific and experimental basis of the principles of microorganism's selection for starter cultures and products**

Currently, in Mongolia, milk processing industries use imported starter cultures for fermented milk, it's quality and safety is not often checked by the competent bodies.

Therefore, a local industry that have starter cultures production ability for fermented milk products is vital for the Mongolian dairy industry.

Ten LAB strains which isolated from traditional dairy products were most promising for dairy industry as follows: *L. paracasei subsp. paracasei (06TSD19), L. paracasei subsp. tolerans (06TSD39), L. delbrueckii subsp. lactis (06DTS3), L. plantarum (05DTS23), L. plantarum (06TSD8), L. paracasei (06TSD22), L. plantarum 06TSD40, L. paracasei (06TSD43), L. plantarum (06LH2), L. plantarum (06LH9)* probiotic properties, and another 4 strains: *Lactobacillus fermentum, Str. Salivarius subsp. thermophilus, L. Helveticus, L.delbrueckii ssp. bulgaricus.*

The microorganisms for developing starter cultures, which most often used in the dairy industry, had been scientifically selected. Strains of lactic acid bacteria were

Streptococcus salivarius subsp. thermophilus TSI/1216, Lactobacillus helveticus TSDI/11, Lactobacillus fermentum DTS/143 they are used for the preparation of the starter cultures.

Dried strains were activated in milk for fermentation. Fermentation was carried out in accordance with the developed method.

Sterilized milk (skim milk) was used. The dried strain powder was dissolved into the skim milk.

An industrial starter was prepared from the mother starter, after fermentation it was cooled and stored at temperature of 0-6 0C.

When preparing bulk starter cultere, in 3%, 5% and 8% sterilized milk of mother starter culture, depending on LAB types.

Fermentation of starter cultures were *Streptococcus salivarius subsp. thermophilus TSI/1216, Lactobacillus helveticus TSDI/11, Lactobacillus fermentum DTS/143* at fermentation temperature of 38, 42 and 45 °C, respectively. The pH was measured in every 2 hours, once reaching to pH 4.6, cooled the starters. Acidity was determined during the fermentation process and the fermentation time was set. Shows the data table 11 on the fermentation time depending on the amount of three types starter cultures.

Table 11. Influence of the type and dose of starter culture in the duration of fermentation process of starter culture

Starter cultures:	Rate of starter culture, %	Incubation time, h		Moment of curd formation	
		2 h	4 h	pH	Incubation time, h
		pH	pH		
Streptococcus salivarius subsp. *thermophilus* TSI/1216	3	6,1±0.04	5,2±0.15	4,7±0.09	5,3
	5	5,1±0.13	4,9±0.05	4,6±0.00	5,1
	8	5,9±0.10	4,8±0.03	4,7±0.08	5,0
Lactobacillus helveticus TSDI/11	3	5,3±0.04	4,7±0.00	4,6±0.01	4,0
	5	4,9±0.05	4,8±0.10	4,6±0.09	3,6
	8	5,0±0.01	4,7±0.30	4,6±0.02	3,4
Lactobacillus fermentum DTS/143	3	5,6±0.02	4,9±0.66	4,7±0.10	5,4
	5	5,3±0.10	5,1±0.48	4,6±0.05	5,3
	8	5,2±0.12	4,9±0.60	4,6±0.01	4,3

As shown in the table 11, the fermentation time of *Lactobacillus helveticus* TSDI/11 had the most activity compared to other strains.

Based on the organoleptic properties of the starter and the time of fermentation, 3 starter cultures were selected for the preparation of the starter culture.

The starter cultures was prepared with various amounts of transfer starter: the first sample of *Lactobacillus helveticus* TSDI/11 was carried in at 3% and 5%, the second sample of

Streptococcus salivarius subsp. thermophilus TSI/1216 - 5% and 8% and the third sample *Lactobacillus fermentum* DTS/143 - 5% and 8% of the total milk volume.

Fermentation was carried out at 38–45 °C. The pH was measured in every 2 hours until reached to pH 4.6, then the starter was cooled. The results were shown in table. 12.

Table 12. Influence of the type and dose of starter culture in the duration of fermentation process of starter culture

Starter cultures:	Rate of starter culture, %	Moment of curd formation	
		pH	Incubation time, h
Streptococcus salivarius subsp. *thermophilus* TSI/1216	5	4,6±0.01	5,5
	8	4,7±0.11	4,9
Lactobacillus helveticus TSDI/11	3	4,7± 0.09	4,1
	5	4,7± 0.01	3,6
Lactobacillus fermentum DTS/143	5	4,7± 0.17	5,3
	8	4,6±0.04	4,3

Starter cultures were added to normalized milk in an amount of 3–8% by volume (depending on the type of starter culture).

Thus, to prepare an industrial starter, it is necessary to add 3–8% of the laboratory starter into sterilized or pasteurized skimmed milk and fermentation for 4–6 hours.

Using of lactic acid bacteria strains with probiotic properties in the fermentation test

The main biochemical and microbiological changes in milk during the producing fermented milk products are caused by microorganisms, which are the part of the starter cultures for these products. Therefore, much attention has recently been paid to the selection of certain strains of microorganisms with probiotic properties (that is probiotics).

Previously, we isolated LAB strains from of Mongolian traditional dairy products. Several of these strains showed tolerance to artificial gastric acids and adhesiveness on human intestinal epithelial cells by *in vitro*, hence these LAB strains were considered probiotic LAB candidates. [Sh. Takeda, Tsend-Ayush Ch. et al., 2011].

Selection of LAB probiotic strains for use in dairy products.

Obviously, probiotics must have activities in intestines. In the present works, studies of LAB isolated from the traditional dairy products of Mongolia were carried out during the fermentation process; pH, lactic acid concentration, and the number of living microorganisms were determined by viable count.

To select a suitable LAB strain for the fermentation of milk among the ten strains, we investigated the curd formation, pH, lactic acid concentration, and numbers of viable bacteria

in skim milk cultivated by each strain. The results of the study were shown in table 13.

Table 13. Comparison of curd formation, acidity, pH, and viable count number in fermented milk with test LAB strains

Lactic acid bacteria	pH	Acidity (lactic acid %)	Viable count number, (log10 CFU/mL)	Curd formation
Blank (skim milk)	6,58±0,02	0,128±0,004	ND	−
L. paracasei subsp. paracasei 06TSD19	4,81±0,26	0,533±0,080	8,9±0,1	+
L. paracasei subsp. tolerans 06TSD39	5,05±0,12	0,453±0,021	8,6±0,1	+
L. delbrueckii subsp. lactis 06DTS3	3,80±0,08	1,091±0,131	8,1±0,2	+
L. plantarum 05DTS23	6,31±0,06	0,157±0,003	7,1±0,1	−
L. plantarum 06TSD8	6,33±0,06	0,159±0,003	7,3±0,1	−
L. paracasei paracasei 06TSD22	5,12±0,10	0,420±0,048	8,1±0,2	−
L. plantarum 06TSD40	6,35±0,05	0,148±0,004	7,3±0,2	−
L. paracasei paracasei 06TSD43	5,25±0,10	0,403±0,048	8,7±0,1	−
L. plantarum 06LH2	6,37±0,05	0,151±0,005	7,2±0,1	−
L. plantarum 06LH9	6,12±0,04	0,183±0,006	7,3±0,1	−

Initial bacterial number: 6.7 ± 0.2 (log10 CFU/mL). The values are expressed as mean ± standard deviation. L., Lactobacillus +, positive; −, negative. ND, not detected.

As shown in the table 13, all the starter cultures were used at the end of fermentation, the viable cell count were 7.1–8.9 log10 CFU/ml. The skim milks inoculated by *L. paracasei subsp. paracasei* 06TSD19 and *L. paracasei subsp. tolerans* 06TSD39 and L. *delbrueckii subsp. lactis* 06DTS3 formed curds and reduced pH values but lactic acid concentrations increased. At the same time, higher lactic acid accumulation ability in for two strains of *L. paracasei subsp. paracasei* (06TSD19, 06TSD39).

In addition, starter cultures prepared by strains of *Lactobacillus paracasei subsp. paracasei* 06TSD19 and *L. paracasei subsp. tolerans* 06TSD39, compared to the other starter cultures, had the largest number of viable cells had a more pleasant taste and aroma, but also had a finer texture.

These results show the feasibility of using a starter prepared by the strain (*L. paracasei subsp. paracasei* 06TSD19), (*L. paracasei subsp. tolerans* 06TSD39 and *L. delbrueckii subsp. lactis* 06DTS3) for the production of fermented products, as known as yoghurt.

The probiotic effects of many LAB strains have been demonstrated. In particular, *L. paracasei, L. casei, and L. rhamnosus*, are genetically related species and popularly used as probiotic LAB [Collins et al., 1991]. Oral administration tests in humans demonstrated that several strains identified as belonging to these species have a strong ability to survive in and

colonize the human gut and improve the human intestinal microflora [Matsumoto et al., 2006; Nishida et al., 2008; Verdenelli et al., 2011].

Only 7 isolated strains of lactic acid bacteria, higher lactic acid accumulation ability selected for the development technology of functional dairy products.

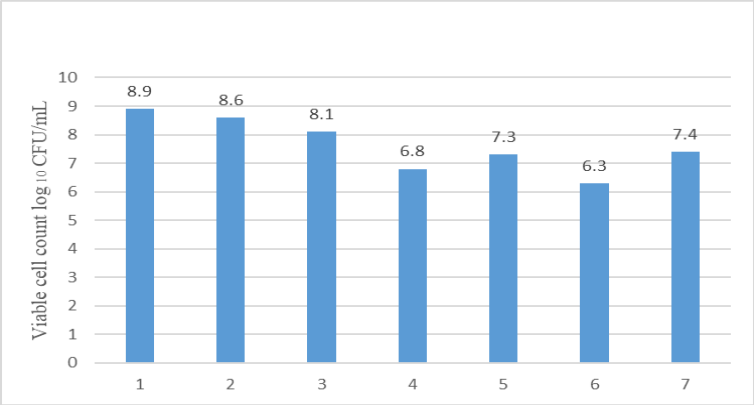

Figure 4. The viable count number lactic acid bacteria (microorganisms) in samples of starter cultures: 1. *L.paracasei subsp. paracasei (06TSD19б); 2. L. paracasei subsp. tolerans (06TSD39б); 3. L. delbrueckii subsp. lactis (06DTS3б); 4. L.fermentum (DTS/143); 5. S. salivarius subsp. thermophilus (TSI/1216);6. L.helveticus TSDI/11; 7. L. Fermentum*

Preparation of combined starter cultures

Our further research was devoted to study and combine a starter culture consisting of different types of lactic acid bacteria, which isolated from tradional dairy products.

In the course production technology of starter cultures using lactic acid bacteria isolated from traditinol dairy products, *L. fermentum* showed the good qualities. Therefore, this culture was chosen for further research for the preparation of all types of combined starter cultures.

Combined starter cultures were prepared from single starter cultures, by a ratios of 1:1; 1:1:1.

Development technology of of functional dairy products based on goat, sheep and cow milk.

One of the outstanding achievements at the end of the 21st century is the development of a fundamentally new concept of “probiotics and functional food”, affecting many fundamental and applied aspects of human health, medicine, nutrition and biotechnology.

In recent years, the problem of healthy food is very vital. Due to a decrease in immunity, various stressful situations, environmental degradation, the population of large cities, including Ulaanbaatar, the of diseases such as atherosclerosis, diabetes, osteoporosis, diseases of gastrointestinal tract and others increased.

In this regard, new functional products is related to Mongolia, since a trend of increasing consumption of functional foods in the world including probiotics, which prevent gastrointestinal diseases and strengthen the general health condition of the body.

Development technology of fermented milk based on goat milk

Currently, probiotic products produced by the basis of bifidobacteria and acidophilus are widely used.

Cultivation of bifidobacteria with lactobacilli, in particular with acidophilus, accelerates the development of bifidobacteria and improves the organoleptic properties of the product.

Combined starter cultures are highly active and resistant to adverse environmental factors in comparison with starter cultures prepared on separate cultures. In this regard, we conducted research on a combined starter consisting of bifidobacterium BB-12 and acidophilus bacillus ABT-2. The optimal combination of cultures in the combined starter was determined taking into account the activity of curd formation, the viable cells, the duration of curd formation, as well as organoleptic characteristics.

As in table 14, the most favorable conditions for the development of bifidobacteria are noted in the third variant (the ratio of cultures is 8:1), where the largest number of viable cells of bifidobacteria was observed.

Table 14. Selection the optimal proportion of culture in the combined starter culture

Ratio of culture	Incubation time, h	Acidity,°T	pH	Viable cell count (log cfu/g)	
				BB-12	ABT-2
2:1	4.5	73-78	4.64	$7x10^7$	$4x10^9$
5:1	6.0	70-75	4.72	$5x10^8$	$3x10^8$
8:1	6.5	60-65	4.80	$3x10^9$	$2x10^8$

At the selected ratio of cultures, the largest number of viable cells of bifidobacteria was observed and the product was characterized by good organoleptic characteristics.

Technology for obtaining a fermented milk product using various types of starter cultures

For preparation of fermented milk products, goat's milk was sterilized at 90-95°C for 10 min, cooled to 37°C, and inoculated with 10% of the bifidobacterium culture, and goat milk was pasteurized at 95°C for 20 min, cooled to 42°C, and inoculated with 3% of the L. acidophilus culture (ABT-2).

During the development of the product, the end of the fermentation process of goat milk was determined by the increase in their acidity to 55 °T, 78 °T, 65 °T – for the with the use of

bifidobacteria, acidophilus bacillus and combined starter, respectively. The duration of milk fermentation for the above starter cultures was 9 hours, 4-4.5 hours and 8-9 hours, respectively.

As known, bifidobacteria and acidophilus have different biochemical activity, which manifests itself in the process of fermentation of milk. Thus, a reduction in the duration of milk fermentation was noted when using acidophilus bacillus compared with bifidobacteria. At the same time, the fermentation time was reduced to 4-4.5 hours and the titratable acidity increased to 78 °T. This is explained with high bio-chemical activity of L. acidophilus. It can be explained due to the higher biochemical activity of acidophilus bacillus.

According to the study results, we can conclude that goat milk is a favorable environment for the development of bifidobacteria, acidophilus bacillus and combined starter.

The physicochemical and microbiological parameters of fermented milk products obtained using three types of starter cultures have been studied.

Finished fermented milk products prepared on the basis of goat's milk, using three starter cultures, are characterized by low acidity and contain a large amount of viabll count. In addition, the products in all three variants had a delicate, uniform texture.

Biological value of fermented milk products

The quality of food products is characterized by their chemical composition, physical properties, as well as nutritional and biological value. Therefore, the biological value is the leading indicator of quality, as it determines the degree of compliance of food products with the optimal human needs according to physiological norms.

As we know, the biological value mainly reflects the quality of the protein components of the products and the level of balance in amino acidity composition.

Amino acid content of fermented milk made by goat’s milk using starter cultures of bifidobacteria BB-12 and acidophilus bacillus ABT-2, as well as combined starter culture are shown in figure 5.

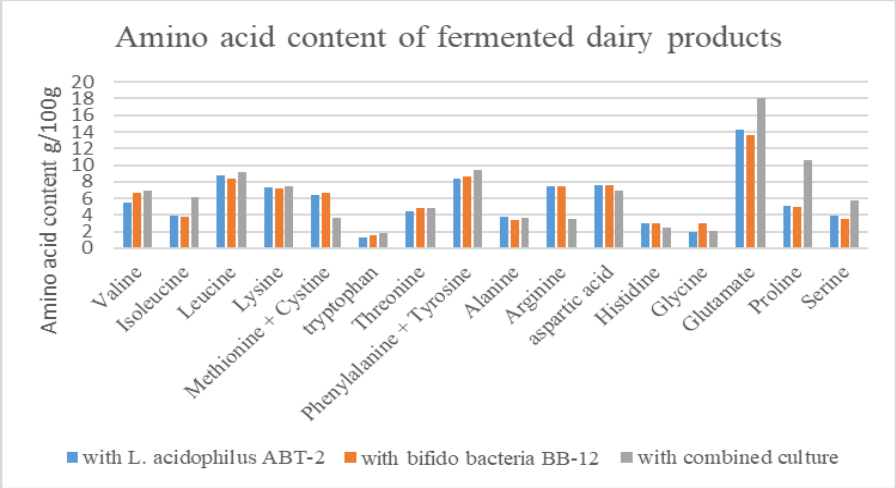

Figure 5. Amino acid content of fermented milk

The data presented in figure 5 show that the fermented milk product using the combined starter is characterized by a higher content of amino acids such as valine, leucine, proline, serine, histidine, isoleicin, tryptophan and glutamic acid.

The biological value of proteins is largely characterized by the composition of the essential amino acids which compared to the "ideal" FAO/WHO protein. To assess the biological value of fermented milk, their amino acid score was calculated, the results are given in table 15.

Table 15. Biological value of fermented milk based on goat milk obtained using other types starter cultures

Amino acid, %	Amino acid score of the fermented milks		
	ABT-2	BB-12	combined culture
Valine	122,6	108,0	133,7
Leucine	143,6	140,5	124,0
Isoleucine	109,6	104,5	145,6
Lysine	134,2	130,1	131,2
Methionine + Cystine	115,1	110,2	118,9
Threonine	133,5	122,6	133,7
Tryptophane	144,3	156,0	124,0
Phenylalanine + Tyrosine	145,2	137,3	145,6

The data presented in the table 15 indicate that fermented milk obtained using all three types of starter cultures are well balanced in terms of the content of essential amino acids and have a high biological value.

According to the study results, we conclude that fermented goat milk products were balanced in their essential amino acid content and these products have a high biological value

Thus, the experimental studies carried out by us made it possible to scientifically substantiate the main technological parameters for the production of fermented milk based on goat's milk.

Development technology of fermented milk using various types of starter cultures

The research allowed to develop a technology for the production of fermented milk products using new various types of starter cultures.

Fermented milk products were prepared according to the traditional technological regulations: dry low-fat cow's milk was used as the raw material. Fresh cream was used to normalize the fat. Normalized milk had the following composition: fat - 3.5%, proteins - 3.1%; lactose - 4.5%; dry matter - 12.3%.

To clarify the technological parameters for obtaining fermented milk products produced with new starter cultures, the strains were isolated from Mongolian fermented milk products, their fermentation process was studied and compared to a control sample produced using the starter culture TCC-3 (Ch.Hansen).

Fermentation was carried out at 40^0C (new cultures) and 42^0C (control**).** Normalized milk inoculated with 5% combined starter culture. pH was measured in every two hours.

The milk was starter culture by *S.thermophilus:L.helveticus*(THM-1)*; S.thermophilus:L.fermentum(TFM-2; S.thermophilus:L.helveticus:L.fermentum*(THFM-3*);) L. delbrueckii lactis : L. fermentum(DLFM-4)*; *S. thermophilus; : L.delbrueckii ssp lactis*(TLDM-5). combined starter culture at temre 40°C; *Str.thermophilus:Lac.bulgaricus* (control) at 42°C. After fermentation yoghurts were cooled down. Results were shown in the table 16.

Table 16. Physicochemical and microbiology characteristics of yoghurts

Composition of products	Characteristics of yogurt, prepared by combained starter culture					
	THM-1	TFM-2	THFM-3	DLFM-4	TLDM-5	TCC-3
Total solids, %	11,8	12,0	11,5	12,1	12	11,7
Fat, %	2,3	2,3	2,4	3,2	3.9	2,6
Protein, %	3,4	3,0	3,3	3,2	3.9	2,7
Lactose, %	3,70	3,7	3,8	3,7	3,7	3,8
pH	4,4	4,4	4,3	4,5	4.3	4,6
Titrable acidity, ^{0}T	82	78	80	82	78	78
Viable count number	$1x10^6$	$8x10^6$	$2x10^6$	$5x10^7$	$3x10^7$	$3x10^6$
ACE	84,29	78,41	59,76	73,72	82.2	76,10
Antoxidant effect, %	5,8	27,80	24,70	12,63	14,5	27,26
Incubation time, h	4-5	4-5	5-6	4-5	4-5	5

Also, for the obtained products, the physicochemical, microbiological characteristics and their sensory evaluation were determined. Tests for angiotensin converting enzyme and antioxidant effect were also carried out.

From the data presented in table 16 the products prepared with starter cultures from lactic acid bacteria isolated from the Mongolian traditional dairy products have a high lactic acid accumulation ability.

Development Technology for soft cheese from goat's milk using microorganizms with probiotic properties

At the present time, the trend of using different dairy raw materials, in particular the wider use of goat milk children's formula, dietary foods and nutritional products are clearly represented around the world. The rational use of goat milk can contribute to the welfare of the rural population through the sale its products of milk at economically justified prices and the sale of its products. With the use of goat's milk, research was carried out on the development technology of soft cheese.

According to the research results, the processing technology of soft cheese made by goat’s milk has been developed.

Technological process of soft cheese production

In the milk preparation- pasteurized at 75 ± 2 °C with an exposure of 3-5 minutes and cooled to 10-12 °C added 0.1 - 0.3% of the starter culture, mix and hold at the specified temperature for 12-24 hours. The titratable acidity of milk at the end of maturation should not exceed 23°T, the active acidity should be 6.3 pH units.

Fat normalization of milk is carried out considering the receipt of a mass fraction of fat in cheese of 45 and 50%, depending on the type of cheese.

Milk is pasteurized at 74±2°C with exposure 3-5 minutes, of the optimal temperature of coagulation at 32-36°C, after then added amount of 1-3% starter culture, 40% calcium chloride solution at the rate of 10-30 g per 100 kg of milk and 1% rennet solution per.

For the development technology of cheese, a culture containing *Lactobacillus paracasei, Lactobacillus. acidophilus* and *Streptococcus thermophilus* were used.

Coagulation period was about 35 to 40 minutes. After the clot is ready it was cut into particles with 20 to 25 mm. At the end of the process, the whey is removed, from 25 to 30% of the whey is removed in 25-30 minutes, then another 25-30% of the whey is removed again (the total removed whey volume is 50-60%.

Cheese after self-pressing is salted with water brine with a concentration of 18-20% at temperature 12-14°C. The duration of salting cheese, depending on the moisture content in cost ~~it, is~~ from 1.5 to 2.5 hours.

After salting, the cheese is dried for 2-3 hours in a room and transferred to a ripening chamber for 3-5 days, while it is turned over 1-2 times.

In fresh soft cheese, which was obtained using a new starter, after pressing, the acidity was 5.15 ± 0.01; moisture content -53.4±0.1%; fat in dry matter -26±0.1%. The results of changes in the physicochemical parameters of the produced soft cheese from goat's milk using a new starter culture at 10–12 °C are presented in table 17.

Table. 17.Changes physicochemical parameters cheese from goat milk in the process of storage

Physico-chemical characteristics	Storage period, day	Starter culture with probiotic properties
Moisture content, %	1	53,4± 0,5
	7	52,9± 0,2
	14	49,2 ± 0,3
pH	1	5,15±0,01
	7	5,10±0,02
	14	4,82±0,02
Fat in dry matter %	1	26,0± 0,2
	7	25,9± 0,3
	14	26,3± 0,2

Since the main difference of the new type of soft cheese is the use of a starter culture with probiotic properties, it was of great interest to study the viable count in the product, which, the average was 8.1±0.2 CFU/g. Viable count of probiotic bacteria remained practically not changed for 14 days, which is an essential indicatorof the quality of soft cheese from goat's milk obtained using a new probiotic starter culture.

Thus, as a result of the studies, rational biotechnological regimes for the production of soft cheese from goat's milk using a new Mongolian probiotics, which was called "Shim", were substantiated and determined. Soft cheese "Shim", made by goat's milk using a new type of starter cultures with probiotic properties, is recommended to be a dietary food product.

Development technology of brine cheese based on sheep's milk

In Mongolia, traditionally, cows, sheep and goats were grazed on mountain pastures in summer, feta cheese is produced by whole cow, sheep and goat milk.

Thus, Mongolia has a large source for the production of cow and sheep milk and it is used in various traditional products, especially pickled cheese, which made by traditional method.

The purpose of the research was to improve the existing technology and develop effective methods for processing sheep cheese into new types of brine cheese.

Obtaining feta cheese with a certain fat content of the cheese mass, the combined milk is normalized, that is high-fat whole sheep's milk is diluted with skimmed cow's milk to obtain 3.5% fat in normalized milk.

Cheese is produced by the acid-rennet method. Sheep milk with an acidity not higher than 22-23^{0}T is filtered, then it is normalized with skimmed cow's milk.

Milk is pasteurized at 70-72°C for 20-30 seconds and cooled to 30-32°C, then a 40% calcium chloride solution added 40±10 g per 100 kg of milk and 1.0 % rennet solution.

In the cheese bath, the mixture is thoroughly mixed, remained for coagulation. Milk coagulation period is at 30-32°C for 30-40 minutes. After coagulation cutting into 7-8 mm, remained for 10-15 minutes and then heated to 38-41^{0}C, mixing for 15-20 minutes until a finished a size of 5-6 mm is obtained.

In salting, the main part of the whey is removed. Fresh cheese is salted in vats with saturated brine.

Brine for salting cheese is made from boiled water. Edible salt is added to hot water until completely dissolved, and then filtered and cooled to 10-12°C.

The establishment of optimal salt and temperature regimes served as the basis for the production of feta cheese.

With such a physico-chemical composition of normalized milk, pickled cheese contains 41.0% fat in dry matter.

Table 18. Chemical composition of cottage cheese “Traditional”

Composition, %	Measured values
Moisture	56,0±1,8
Fat	18,0±0,4
Protein	19,0±0,5
Minerals	4,0±0,1
Organic acid	3,0±0,1

Development technology of yoghurt with probiotic properties

For technological research on the development technology of new types of yoghurt with probiotic properties we use cow's milk.

For the development of yoghurt technology *L. paracasei* strain isolated from traditional dairy products and functional ingredients (welding protein, glucose and gelatin) were used as the main starter culture.

To increase the biochemical activity of the starter during fermentation and directed regulation of microbiological processes in the production of fermented milk products, it is advisable to use certain growth stimulants.

All additives, including milk, welding protein, glucose and gelatin were added in the same amount.

Ingredients can be added to milk before pasteurization, or to hot milk after pasteurization, or to a milk clot after fermentation, homogenization at a pressure of 15.0 MPa and a

temperature of 65ºC, pasteurization at a temperature of 90–92ºC and cooled to fermentation temperature 37 ºC. Bacterial starter on pure cultures was introduced into the prepared milk mixture.

The samples of yoghurt were produced according to the traditional technology in a thermostatic way.

The end of this process is determined by the formation of a dense clot and the achievement of a titratable acidity of 75–85°T. After fermentation cooled produst to temperature. 10–12 ºC.

The finished product was examined for organoleptic and microbiological parameters when stored in a refrigerator 6-8 °C) for 10 days to determine its shelf life. According to the results, the number of viable microorganisms in the fermented milk product was found to be 10.9 CFU/ml on the first day of storage, and 10.9 CFU/ml on the 7th day. No change in organoleptic parameters was observed during 7 days of storage. Therefore, the shelf life of the product is limited to 7 days, which, subject to storage conditions, guarantees a high viable count number of probiotic microorganisms and good rheological characteristics.

Development technology of curd product

Curd product are traditional product rich protein, which are the most in demand as part of the growing interest of the population in a healthy diet. Its nutritional and biological value is due to the high content of amino acids, including sulfur-containing ones - methionine and leucine, as well as choline, calcium, phosphorus, etc.

The development of curd products with probiotic properties involves starter cultures containing *Lactobacillus* as part of the microflora. *L.helveticus* and *L. delbrueckii subsp. lactis* in a 1:1 ratio.

During the research, it was found that for the technology of the milk-protein basis of curd product, it is necessary to use the following production parameters: Milk was pasteurized at a temperature 86 ± 2 °C for 20 seconds.

After pasteurization, it was cooled to a temperature (32 ± 1°C) and $CaCl_2$ was added in the form of a 40% water solution and the starter in an activated form. The starter is added in the amount of 5% to the volume of the fermented milk base.

Coagulation was carried out by adding a 10-15% solution of calcium chloride at a temperature of 33-34^{0}C.

Two strains of lactobacilli were used as cultures for fermentation: The starter culture DC-50 (*L. helveticus: L. delbrueckii subsp. Lactis,* isolated from Mongolian traditional dairy

products) includes probiotic cultures of *Lactobacillus delbrueckii subsp. bulgaricus.* The starter was inoculated in an activated form. Activation was carried out on sterilezed skimmed milk.

Coagulation is carried out at the current temperature until a sufficiently strong clot with acidity 80±2°T is formed (the duration of fermentation is 8-9 hours.)

The method of curdling to obtain the base curd is acidic. All procedures were carried out in 5-fold repetition, the results were analysed using mathematical statistics.

Then the clot was subjected to processing in order to separate the whey (cutting, holding) and heating to a temperature of 40 ± 2 ^{0}C to separate the whey, with stirring and gradual separation of the whey for 60-90 minutes. Self-pressing at a temperature 20 ± 2°C for 1.5 hours until a clot with a mass fraction of moisture 65-70% is obtained and cooling to a temperature of 10-12°C.

For the composition of the curd product, whey proteins and sea buckthorn juice were chosen.

In order to establish a rational dose of introducing whey proteins into curd whey, the influence of its mass fraction on the organoleptic and rheological properties and consistency of the product was studied. Whey protein of curd whey was added in the amount of 20%.

Seabuckthorn is one of the most valuable natural sources of water- and fat-soluble vitamins and vitamin-like compounds; organic acids, mineral and other substances.

For the preparation of seabuckthorn syrup, sea buckthorn juice and sugar were used, drinking water heated to a temperature of 40–45 °C, and then the mixture was filtered. The pasteurization of the mixture was carried out at a temperature of 85 ± 2°C with a holding time of 15 minutes and then cooled to 8–10°C.

To improve the taste of the product, it is proposed to use berry fillers (seabuckthorn syrup) in the amount of 18-20% of the total volume of the product.

Table 19. Physico-chemical and microbiological characteristics

Quality parameters	Measured values
Fat, %	18
Protein, %	15
Lactose, %	10-15
Titradable acidity, ^{0}T	150-170
Moisture, %	76-78
Viable count (Log 10 CFU/g)	10^8
Escherichia coli	-

The physico-chemical and microbiological characteristics and product safety indicators, as well as the vitamin composition of the enriched curd product were studied.

The biological value of curd products was studied, reflecting the quality of the protein component and the balance of its amino acid composition.

To characterize the biological value of protein products, the amino acid score method was used, which represents the ratio of the actual indicator of the amount of essential amino acids to its content in the "ideal protein".

Table 20. Acid acid score of curd product

Amino acid	Amino acid score			
	FAO/WHO benchmark		Curd product	
	А	Б	А	Б
Lysine	5,5	100	7,9	148
Threonine	4,0	100	4,3	109
Valine	5,0	100	6,0	170
Isoleucine	4,0	100	5.8	145
Leucine	7,0	100	9,2	131
Methionine + Tryptophane	3,5	100	4,9	138
Phenylalanine+ tyrosine	6,0	100	8,6	143
Tryptophane	1,0	100	1,5	150

Note: A- mass fraction of an irreplaceable amino acid, %;
B- Amino acid score, % reference scale FAO/WHO

Analysis of the data presented in table 20 allows us to conclude that the product does not contain limiting amino acids, i.e. the product belongs to biologically high-grade dairy products.

And also studied microbiological indicators and indicators of product safety.

Development technology of yogurt added green olive powder

Technologies for green olive powder containing yoghurt have been developed, technological characteristics, content of polyphenolic compounds and antioxidant activity of the product have been studied.

Skimmed milk, pectin, sugar and green olive powder at 0%, 1%, 3% and 5% were homogenized for 5 minutes.

The mixture was sterilized at 85°C for 30 minutes and then cooled to 42°C in a water bath. After inoculation the culture of lactic acid bacteria, the mixture was incubated at 37°C for 8 hours until the pH of the medium reached 4.5. After fermentation, all yogurt samples were stored at 4°C in a refrigerator. Samples were collected at interval of 5 days for 15 days for analysis (1, 5, 10 and 15 days).

(GY0: control), 1%, (GY1:1% green olive yogurt), 3% (GY3: 3% green olive yogurt) and 5% (GY5: 5% green olive yogurt) were homogenized for 5 min.

Lactic acid bacteria including *Streptococcus thermophilus, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus acidophilus and Bifidobacterium animalis ssp. Lactis* were used as starter culture for yogurt.

Results of measuring the pH and titratable acidity of yoghurt samples with the addition of green olive powder during storage at 4°C for 15 days.

The pH value of yogurt after finishing fermentation ranged from 4.38 to 4.41. After that, incipient pH of storage ranged from 4.44 to 4.55. GY5 treatment resulted in no significant change in pH after storage. During storage, the pH of the GY5 sample did not change.

The decrease of pH during the storage might be due to accumulation of lactic acid by metabolic activity of bacteria (Tseng and Zhao, 2013).

According to Lee and Hwang (2006), the optimum pH of thick fermented milk coming into the market is from 3.27 to 4.59. In this study, after storing yogurt for 15 days at 4°C, the pH also fell into this optimum range. This mean that the quality of yogurt added green olive yogurt is not different from the quality of fermented milk in the market.

The initial titratable acidity values of yogurt ranged from 0.92 to 0.94%. After storage, titratable acidity values were increased in all treatment groups. After 15 days of storage, titratable acidity values ranged from 1.07 to 1.14%. Results of the viable count number of lactic acid bacteria in yogurt stored at 4°C for 15 days are shown in table 21.

Table 21. Lactic acid bacteria counts (Log CFU/g) during storage of yogurt added green olive

Storage period (d)	GY0	GY1	GY3	GY5
1	9.38 ± 0.80	9.18 ± 1.32	9.00 ± 0.98	8.95 ± 0.37
5	9.33 ± 0.29	9.13 ± 0.53	8.95 ± 1.37	8.94 ± 0.57
10	9.15 ± 0.12	8.74 ± 0.30	8.64 ± 0.17	8.55 ± 0.15
15	8.65 ± 0.03	7.82 ± 0.24	7.72 ± 0.02	7.69 ± 0.20

1)GY0: control; GY1: yogurt added with 1% green olive; GY3: yogurt added with 3% green olive; GY5: yogurt added with 3% green olive.

2)Means with different deviation for three replicate superscripts (A, B in the same column and a, b in the same row) differs significantly (p<0.05). All values are means ± standard deviation for three replicates.

The number of initial lactic acid bacteria in all groups was over 9.0 Log CFU/g. The number of lactic acid bacteria in yogurt after storage for 15 days was decreased to 7.7-8.7 Log CFU/g.

Such viable count number of lactic acid bacteria in all groups after 15 days of storage was high enough compared to standard yogurt.

Total polyphenol content (TPC) and antioxidant activity

Results of total polyphenol contents and antioxidant activity in yogurt after storage at 4°C for 15 days.

On the first day of storage, the content of phenolic compounds in the GYO, GY1, GY3, and GY5 samples was 4.30, 4.51, 5.85, and 6.96 mg GAE/kg, respectively in the 1st day. During storage of yoghurts, the content of polyphenolic compounds decreased TPC ($p>0.05$). This result was consistent with results of a previous study showing that TPC values of yogurt added with grape and callus extracts were decreased when storage period was longer [Karaaslan et al., 2011]. Temporary decrease of TPC in yogurt could be decomposition of polymeric phenolics in the presence of lactic acid bacteria during refrigerated storage [Dalling, 1986].

However, on day 15 of storage, reducing power values of GY0, GY1, GY3, and GY5 were not significantly different. On the 15th day of storage, the reducing ability of GYO, GY1, GY3 and GY5 did not differ significantly.

DPPH radical scavenging activity of GY5 and GY3 were at 47% and 44% each, which was the highest among all groups. After 15 day of refrigerated storage, DPPH radical scavenging activities of GY0, GY1, GY3, and GY5 groups were decreased to 21%, 26%, 27%, and 29%, respectively. This is consistent with results of a previous study showing that DPPH radical scavenging activity of yogurt added with grape and callus extracts yogurt after storage of 14 days is decreased 1.16-3.78 times [Karaaslan *et al.*, 2011].

It has been reported that green olive has plenty of polyphenol compounds such as oleuropein [Amiot et al., 1986].

In addition, the hydrolysis of milk proteins and the production of organic acids affect the antioxidant activity of yogurt with the addition of green olive powder.

Antioxidant activity in green olive powder added yogurt was higher than that in GY0 during 15 days. Yogurt with the addition of 3% green olive powder has a better antioxidant effect than GY0.

Investigation of sensory characteristics

Sensory test of the manufactured yogurt was implemented through acceptability test after different amounts of green olive powder was added to yogurt.

Taste evaluation of samples GY0, GY1, GY3 and GY5 (storage at 4°C) was performed by 30 trained panelists after 3 days of making yoghurt.

The studies were performed in triplicate and were analyzed by one-way analysis of variation using SPSS/PC Statistics 18.0 software (SPSS Inc., Chicago, IL, USA).

After 10 days of storage, the viscosity of the GY1 sample did not differ significantly from the initial one. After 5 days of storage, the viscosity of samples GY1, GY3, and GY5 was higher than GYO ($p>0.05$).

Over time, the viscosity of GY5 decreased in GY3, no significant difference was observed within 5 days, but after 10 days of storage, the viscosity decreased ($p<0.05$). GYO and GY1 had no significant changes after 5 days of storage.

Enriching yoghurt and dairy products with fiber improves quality and promotes health. GY3 and GY5 had a higher viscosity compared to GYO and GY1 when stored up to 5 days. Viable lactic acid bacteria in the samples did not differ significantly within 10 days, with the exception of the GY5 sample. All samples supplemented with green olive powder had high antioxidant activity values compared to the GYO control.

Sensory evaluation results of yoghurt samples with different amounts of green olive powder additive, GY3 yogurt color value was 4.63, which was the highest among all experimental groups, GY0 had the highest sour score. Flavor scores of GY0 and GY3 were 4.53 and 4.25, respectively, without significant difference between the two. Sweetness score of GY3 was 3.06 which was the highest among all groups. The taste score of GYO and GY3 did not have a significant difference, these samples were rated 4.53 and 4.25 points. The sweetness of the GY3 sample received the highest score of 3.06. The overall GY0 and GY3 scores were 3.94 sensory scores.

However, when green olive powder is added to yogurt, lactic acid bacteria tend to be decreased more than control yogurt after 10 d of storage.

All samples with the addition green olive powder had high antioxidant activity compared to the GYO control.

This study showed that yogurt added 3% of green olive produced the acceptable product that influenced to substantial helpful health. Results of this study demonstrated that green olive powder might be used to improve the antioxidant capacity and sensory characteristics of yogurt.

Development technology of synbiotic dairy product

The production of food products enriched with functional ingredients for the prevention of diseases and the improvement of the population of environmentally unfavorable regions is in the center of attention of world science.

Prebiotic nutrition products that contain prebiotic ingredients, which often add nutritional value to foods.

Inulin and oligofructose are commonly used as the most important prebiotics

(Oliveira et al., 2009a). Synbiotics may provide more beneficial effects than individual probiotics or prebiotics. For the combination of prebiotics, inulin and FOS will be used.

In the study, inulin and FOS will be combined with lactic acid bacteria strains for use in the development technology of synbiotic product.

In the experiment, an inulin preparation was used, which added at different concentrations of inulin (1.5, 2 and 2.5%) to the samples of fermented milk products and starter cultures with probiotic properties.

For the preparation of products, milk with a fat content of 2.5% and solids of 8.6% was used.

The milk was heated to 40 °C, inulin was added and thoroughly mixed until dissolved. Before pasteurization, we add inulin, in the amount of 1.5, 2 and 2.5%.

A sensory evaluation of synbiotic yoghurt with various concentrations of inulin was carried out and the results of the evaluation show that synbiotic yoghurt, the addition of inulin at a concentration of 2%, can significantly increase the viscosity of the mixture and improve the consistency of the product.

To produce synbiotic yoghurt, pasteurized milk (2.5% fat) and were inoculated starter culture of *Streptococcus thermophilus, L. paracasei subsp. tolerans and Bifidobacterium longum* (2:1:1) and added inulin and FOS. Incubated at 40 °C. When the pH value of the samples reached 4.5 – 4.7, they were refrigerated. Synbiotic yoghurt was storaged the refrigerator at 4℃ for 14 days.

The developed technology of synbiotic dairy products and the physical-chemical, microbiological and sensory characteristics of product milk were studied.

Table 22. Physical-chemical, characteristics of synbiotic dairy products

Physico-chemical characteristics	Measured values
Fat, %	2.5
Dty matter, %	14.6
Acidity, ºT	78
Viscosity, 4^0C mPa**.**s	1560-1561

Vitamins A, E, B_1, B_2, C of synbiotic dairy productswere determined (Table 23).

Table 23. Content of synbiotic yoghurt

Synbiotic yoghurt	Vitamins, mg/ 100g				
	B_1	B_2	A	E	C
	17,2	109,9	21,7	12,5	1,8

pH and titratable acidity, and the number viable count were determined every 7 days, shown in the table 24.

Table 24. Changes pH, titratable acidity and viable count of product during 14 days of storage

Physico-chemical parameters	Day		
	1	7	14
pH	4.45±0.02	4.31±0.03	4.27±0.01
Acidity, ^{0}T	82±1.1	88±1.2	93±1.3
Viable count number	8.42	8.38	8.15

The viable count number of probiotic bacteria increased up to the first week and then decreased. The amount of probiotic bacteria in the synbiotic yoghurt was above 8.9 log CFU/ml. When stored in a refrigerator, the pH of the synbiotic yoghurt decreased, while the viscosity increased. Also, syneresis was decreased in 14 days, and then was increased.

In this study it was shown that the acidity and the percentage of syneresis have increased with regard to the growth and the activity of starter and probiotic bacteria. The addition of different percentages of prebiotics resulted in slowed trend of variations and improved the quality of the product**.**

Y. Research results *In Vitro* and *In Vivo* Anti-*Helicobacter Pylori* Activity

V.1 *In vitro* and *in vivo* study of anti-*Helicobacter pylori* activity

Effects of LAB on H. pylori growth in vitro. In screening the tested LAB for anti-*H. pylori* activity, LAB were co-cultured with *H. pylori* No. 130 for 2 days, and *H. pylori* counts were subsequently determined colony count method.

All of the tested LAB strains demonstrated a decrease in the growth ratio of H. pylori. In particular, significant differences were observed in the growth ratio of H. pylori co-cultured with *L. plantarum* strains 05AM23 (37.3%) and 07MR044 (35.4%) compared to PBS (control). For *L. paracasei*, strain 06TSD19b showed the lowest *H. pylori* growth ratio (46.2%), but a statistically significant difference was not observed in comparison with other studied strains.

Thus, inhibition of growth of H. pylori by lactic acid bacteria depends not only on the type of microorganism, but also on the particular strain used. The results of our experiments revealed the antibacterial effect of strains 06TSD19b and 07MR044 belonging to the species

L. paracasei and L. plantarum, respectively. The growth ratio of *H. pylori* in the co-cultures is shown in table 25.

Table 25. Tested LAB and their effects on the growth of *H. pylori.*

Species	Strain	Growth ratio of H. pylori (%)
06TSD19b	*Lactobacillus paracasei* subsp. *paracasei*	46,2 ± 11,2
06TSD22b		56,8 ± 19,0
06TSD43b		49,3 ± 11,8
06TSD39b	*Lactobacillus paracasei* subsp. *tolerans*	53,5 ± 15,0
07LHD080c		57,1 ± 22,7
08LFH34d		59,5 ± 18,0
08LFH65c		86,1 ± 6,4
08LFH75c		66,2 ± 5,8
05DTS23c	*Lactobacillus plantarum*	37,3 ± 2,9*
06LH2d		48,6 ± 4,4
06LH9d		41,4 ± 3,6
06TSD8b		41,6 ± 4,8
06TSD40b		42,6 ± 10,0
07MRD44b		35,4 ± 7,3*
08MRD29e		61,7 ± 2,7
06DTS3b	*Lactobacillus delbrueckii* subsp. *lactis*	61,3 ± 11,9
PBS (фосфатно-солевой буфер)		100,0 ± 8,1

Data represent mean ± standard deviation (SD).

The experiment was independently carried out three times. The asterisks indicate significantly differences from the control (PBS) by one-way ANOVA, followed by Tukey's test ($p < 0.05$).

[a]Growth inhibition ratios were expressed as a percentage of the control. H. pylori strain no. 130 counts in coculture with each LAB were determined on modified Skirrow's agar.

[b]LAB strains were isolated from tarag, a traditional Mongolian yogurt.

[c]LAB strains were isolated from airag-fermented mare's milk.

[d]LAB strains were isolated from aaruul, a traditional Mongolian cheese.

[e]LAB strain was isolated from urum, a clotted cream made from milk. The tested LAB strains were isolated from ninety-five tarags, thirtyone airags, nine aaruuls and two urums.

Potent growth inhibition of *H. pylori* by strains 06TSD19b and 07MR044 was noted among *L. paracasei* and *L. plantarum* species, respectively, in the screening process; therefore, these strains were used in subsequent experiments.

To compare the H. pylori growth-inhibition abilities of strains 06TSD19b and 07MR044, the initial counts of LAB and H. pylori in the co-culture were adjusted to 6.0 log10 CFU/mL, and bacterial counts and medium pH were estimated during the time course of co-culturing. The research results are shown in Fig. 6.

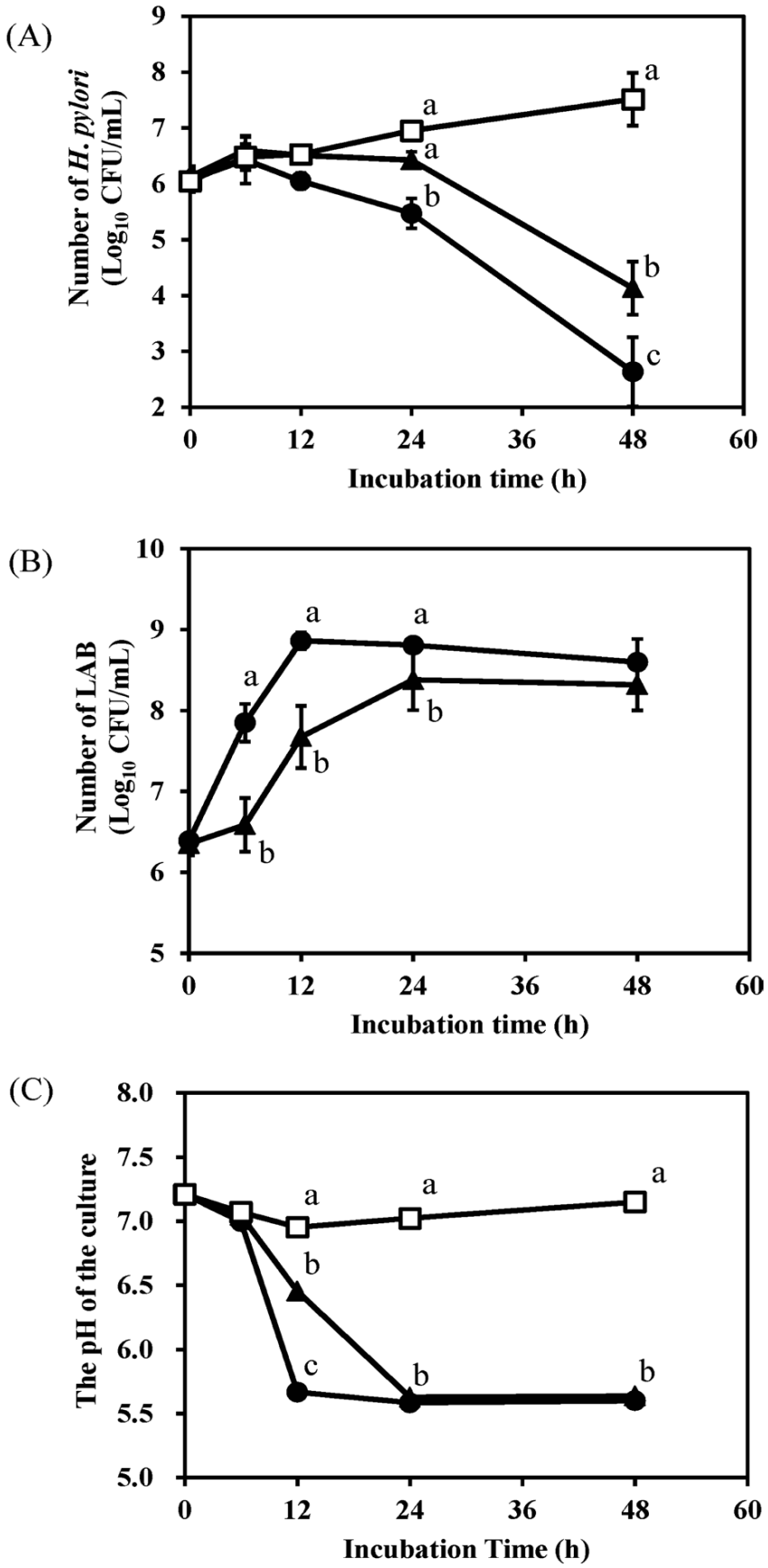

Figure 6. Effects of LAB in co-culture with *H. pylori*.

(A) The count of *H. pylori* in co-cultures with the strain 06TSD19б (●), 07MR044 (▲), and PBS (□).

(B) The count of LAB in the co-cultures of *H. pylori* strain no. 130 with the strain 06TSD19б (●) and 07MR044 (▲).

(C) pH in the co-cultures of *H. pylori* strain no. 130 with the 06TSD19b strain (●), 07MR044 strain (▲), and PBS (□).

The experiments were independently carried out three times. Vertical bars represent SD. Values with different small letters indicate a significant difference at each incubation time by two-way ANOVA, followed by the Bonferroni's test ($p < 0.05$).

The counts of *H. pylori* co-cultured with strain 06TSD19b were significantly lower than the control and with strain 07MR044 at 24 h and 48 h incubation ($p < 0.05$, two-way ANOVA, followed by Bonferroni's test).These studies are shown in Figure 6. Therefore, it was considered appropriate to conduct further studies with these strains.

The LAB counts at 6 h, 12 h and 24 h incubation were significantly higher, and pH at 12 h was significantly lower, in co-culture with strain 06TSD19b than with strain 07MR044 (Fig. 6B and 6C).

In addition, the pH of the medium was lower when H. pylori was co-cultivated with the 06TSD19b strain. A correlation was observed between a decrease in the *H.pylori* counts and the pH of the medium, which in turn affected the increase in the count of lactic acid bacteria of the 06TSD19b strain.

Thus, inhibition of the growth of H.pylori during co-cultivation with strain 06TSD19b may be associated not only with the action of organic acids, but also with the release of certain metabolites that affect the growth of microorganisms

Identification of the active anti-H. pylori component(s) in LAB Culture

The culture solutions of strains 06TSD19b and 07MR044 and their supernatants exhibited fairly large inhibitory zones against H. pylori (Table 26).

However, the fractions of pH-neutralized supernatant and pH-neutralized supernatant treated with catalase showed significant smaller inhibitory zones that in culture solutions and the untreated supernatants.

The inhibitory zones from the fractions of 06TSD19b and 07MR044 cells were also significantly smaller than those of the fractions of the culture solutions or the untreated supernatants. In addition, heat-killed 006TSD19b and 07MR044 cells did not produce inhibitory zones, respectively.

The supernatant fractions of strains 06TSD19b and 07MR044 exhibited high inhibitory activity against H. pylori (Table 26).

As can be seen from the results of the experiment shown in tables 26, 27, neutralization of the pH of the medium and treatment with catalase leads to a significant decrease in the inhibitory effect of strains 06TSD19b and 07MRD44b on the growth of Helicobacteria. Therefore, the inhibitory activities of strains 06TSD19b and 07MR044 against *H. pylori* were thought to be caused by the production of organic acids. Also, the level of organic acids, including lactic acid, produced by the LAB strains that did not inhibit H. pylori growth (Table 26) may be lower than that produced by strains 06TSD19b and 07MRD44b.

Table 26. Effect of LAB culture fractions on *H. pylori* growth.

Fraction of LAB culture	Diameter of the inhibition zone (mm)	
	06TSD19б	07MRD44б
PBS	ND	ND
Culture solution (containing cells)	17,1 ± 2,3a	18,0 ± 0,2a
Supernatant	17,7 ± 1,8a	18,7 ± 2,8a
Supernatant pH neutralized to 6.5	3,9 ± 0,9б	2,7 ± 0,7б
Supernatant pH neutralized to 6.5 and treated with catalase	2,9 ± 1,1б	1,6 ± 0,6б
LAB cells	2,8 ± 0,8б	2,2 ± 0,1
Heat-killed LAB cells	ND	ND

Samples were prepared from three independent experiments and subjected to a well diffusion assay. Data represent the mean ± SD. ND-not detected. a, b – statistically significant difference according to one-way ANOVA experiment using Tukey's test ($p < 0.05$).

Analysis of organic acids in co-cultured H. pylori and LAB

The organic acid levels in the supernatants of co-cultured *H. pylori* and LAB were analyzed. The lactic acid concentrations in the co-cultures of *H. pylori* and LAB increased in a time-dependent manner, but no lactic acid was detected in the supernatants of PBS (control) (Table 26).

Table 27. Levels of organic acids in the co-cultures of LAB and H. *pylori.*

Strains	Lactic acid (mmol/L)				Acetic acid (mmol/L)			
	6 h	12 h	24 h	48 h	6 h	12 h	24 h	48 h
PBS	ND	ND	ND	ND	72,9 ±2,3	68,2 ± 3,3	69,4 ± 2,9	69,8 ± 2,8
06TSD19б	9,0 ± 1,0	25,0 ± 0,5*	24,5 ± 0,6	23,3 ± 0,3	71,2 ± 2,6	73,4 ± 3,4	74,8 ± 4,9	79,3 ± 2,6
07MRD44б	8,7 ± 1,1	15,1 ± 2,2	22,6 ± 2,5	22,2 ± 1,5	69,6 ± 2,2	66,8 ± 2,5	71,0 ± 2,1	72,8 ± 5,3

Samples were collected from three independent experiments for analysis by HPLC. Data represent the mean ±SD. ND, not detected. Levels of lactic and acetic acids in the initial incubation were ND and 63.2 ± 2.0 mmol/L, respectively. Lower limits of detection were 2.5 mmol/L for lactic acid and 12.4 mmol/L for acetic acid. The asterisk indicates significantly different from the lactic acid level of strain 07MR044 at 12 h by two-way ANOVA, followed by Bonferroni's test ($p < 0.05$).

The concentration of lactic acid in the co-culture with strain 06TSD19b reached 25.0 mmol/L at 12 h incubation, and the lactic acid concentration was significantly higher than that in the co-culture with strain 07MR044 at 12 h. On the other hand, no significant differences were observed in the concentrations of acetic acid of the control and co-cultures inoculated with the LAB strains.

As for the 06TSD19b strain, compared to the 07MRD44b strain, it not only more effectively suppresses the growth of *Helicobacter pylori*, but also more intensively reduces the acidity in the medium. When determining organic acids, an analysis of the co-cultivation of the supernatant of H. pylori no. 130 with strains 06TSD19b and 07MRD44b was carried out. High antihelicobacter activity was revealed in strains 06TSD19b and 07MRD44. It has been established that organic acids formed as a result of the development of strains 06TSD19b and 07MRD44 inhibit the growth of *H. pylori* No. 130 more intensively than the bactericions of these strains.

As shown in Table 27, the increase in lactic acid concentrations in the co-culture of *H. pylori* and LAB was due to the growth of LAB, as no lactic acid was detected in the control. In addition, the lactic acid concentration of the co-culture inoculated with strain 06TSD19б was significantly higher than that with strain 07MR044 at 12 h after LAB inoculation ($p < 0.05$).

Thus, strain 06TSD19b produced a larger amount of lactic acid compared to strain 07MR044 during early-stage co-culture with *H. pylori*. This ability of 06TSD19b may contribute to the anti-*H. pylori* activity.

Acetic acid was detected in the *H. pylori* culture inoculated with PBS, and no significant differences were observed in the concentrations of acetic acid in this study (Table 27). Therefore, it is suggested that acetic acid was not produced by strains 06TSD19б and 07MR044 and did not contribute to the inhibition of *H. pylori* in this study.

Effects of lactic acid produced by LAB during early-stage co-culture on H. pylori growth to investigate the effect of lactic acid produced by strains 06TSD19б and 07MR044 during early-stage co-culture on *H. pylori* growth, 25 mmol/L L-lactic acid and 15 mmol/ L DL-lactic acid were added to the *H. pylori* culture at 12 h incubation, and *H. pylori* was enumerated. The concentrations and schedules for L and DL -lactic acid addition were based on the results given in table 27, which showed the inoculation of strains 06TSD19б and 07MR044 to *H. pylori* cultures, respectively. It was also confirmed that strains 06TSD19b and 07MR044 produced L-lactic acid and DL-lactic acid, respectively

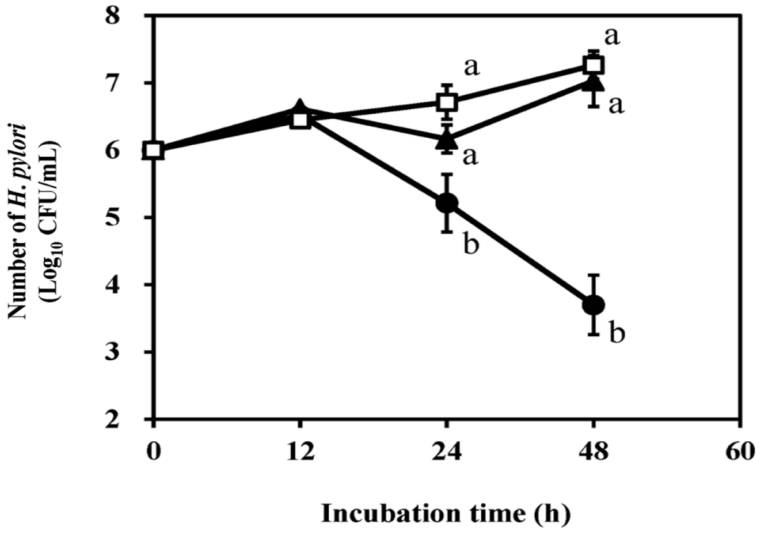

Figure7**.** Effect of lactic acid on *H. pylori* counts.

H. pylori strain no. 130 counts in cultures inoculated with 25 mmol/L L-lactic acid (●), 15 mmol/L DL-lactic acid (▲), and PBS (□) at 12 h incubation are shown. The experiment was independently carried out three times. Vertical bars represent SD. Values with different small letters indicate a significant difference at each incubation time by two-way ANOVA, followed by Bonferroni's test ($p < 0.05$).

As shown in Fig. 7, the addition of 25 mmol/L L-lactic acid at 12 h incubation significantly reduced *H. pylori* counts in the medium to lower than that observed with PBS or 15 mmol/L dl-lactic acid addition at 24 h and 48 h incubation ($p < 0.05$, two-way ANOVA, followed by Bonferroni's test). On the other hand, the addition of 15 mmol/L DL-lactic acid did not produce a time-dependent reduction in *H. pylori* counts.

The anti-*H. pylori* activity mediated by lactic acid has been described in previous reports [Midolo *et al*., 1995; Zheng *et al*., 2014]. The effect of lactic acid on *H. pylori* strain was also determined in this study. As shown in Fig. 7, the addition of 25 mmol/L L-lactic acid at 12 h incubation significantly reduced *H. pylori* counts as compared to the addition of 15 mmol/L dl-lactic acid at the same time-point or the control. This result was consistent with the notable reduction in *H. pylori* counts upon inoculation with strain 06TSD19 *in vitro* (Fig. 6A). Also, *H. pylori* growth inhibition mediated by the activities of D and L-lactic acids was found to be similar (IC50 of approximately 23.0 mmol/L for both).

Therefore, the large amount of l-lactic acid produced by strain 06TSD19b during early-stage co-culture was inferred to contribute to the growth inhibition of *H. pylori*.

The large amount of L-lactic acid produced by strain 06TSD19b is expected to inhibit the adhesion of *Helicobacter pylori to* human gastric cells.

Effects of oral administration of LAB on bacterial counts in the stomach of H. pylori-infected mice.

To investigate the effects of strains 06TSD19b and 07MR044 on *H. pylori* colonization in the stomach of mice, infected mice were orally administered the respective strains. *H. pylori* counts in the stomach of infected mice administered strain 06TSD19b were significantly lower than in those administered PBS (control) or strain 07MR044 ($p < 0.05$, one-way ANOVA, followed by Tukey's test) (Fig. 6A). Further, no reduction was noted in *H. pylori* counts in the stomach of infected mice administered strain 07MR044. On the other hand, LAB counts in the stomach of infected mice administered strain 06TSD19b were higher than in those administered strain 07MR044, although not significantly so (Fig. 6B).

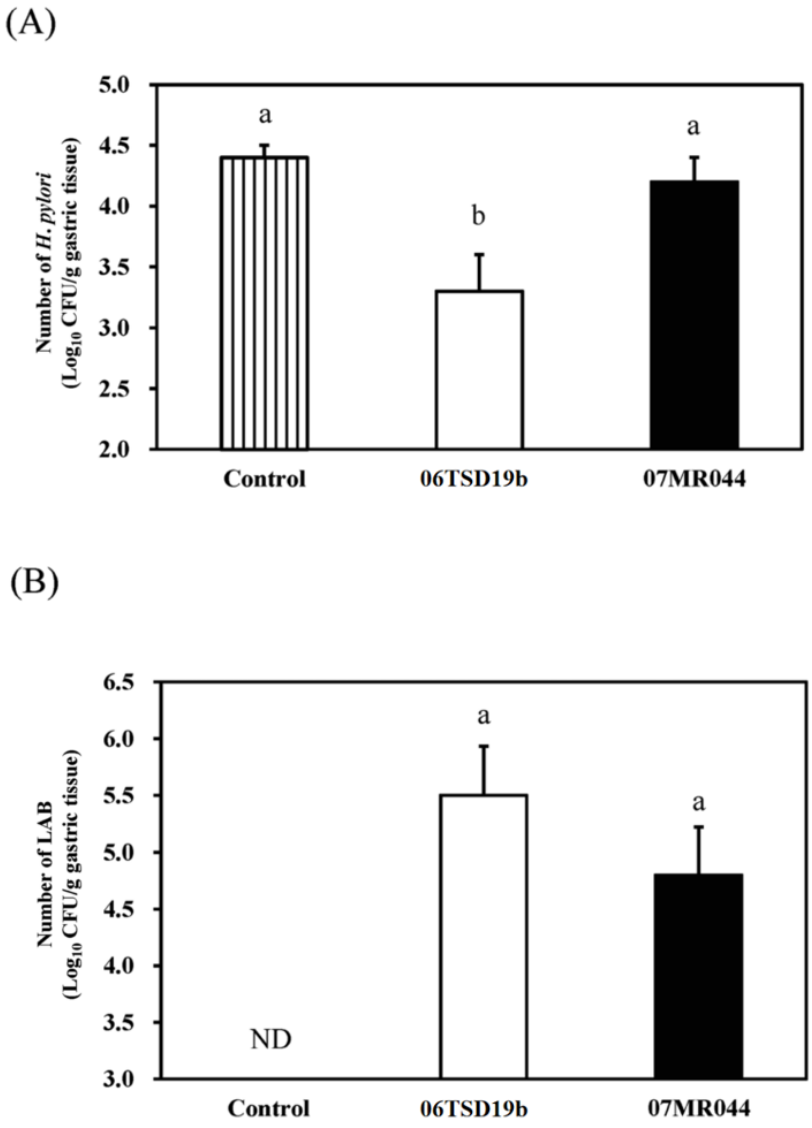

Figure 8. Effects of oral administration of LAB on bacterial counts in the stomach of *H. pylori*-infected mice.

(A) *H. pylori* counts in the stomach of *H. pylori* strain no. 130-infected mice (n = 7). Striped, hollow, and solid columns show the numbers of *H. pylori* in the stomach of *H. pylori* strain no. 130-infected mice administered PBS, strain, and strain 07MR044, respectively. (B) LAB counts in the stomach of infected mice (n = 7). Hollow and solid columns show the numbers of LAB in the stomach of infected mice administered strain 06TSD19b and strain 07MR044, respectively. Vertical bars represent standard error (SE). Values with different small letters indicate a significant difference by one-way ANOVA, followed by Tukey's test ($p < 0.05$). ND indicates the lower limit of detection (< 2.0 $\log_{10}$ CFU/g).

In this study, oral administration of strain 06TSD19b to infected mice produced a significant reduction in *H. pylori* levels, and the strain 06TSD19b showed a beneficial effect on reducing or eliminating H. pylori in the stomach *in vivo.*

The beneficial effect of *L. salivarius* WB1004 administration on the eradication of *H. pylori* in the stomach of infected mice was due to its ability to adhere to gastric epithelial

cells and produce high levels of lactic acid, in parallel with its rapid growth in the stomach [Aiba *et al*., 1998]. In addition, *L. gasseri* OLL2716 showed strong anti-acid properties and tolerance to gastric juices *in vitro*, leading to the suppression of *H. pylori* infection [Fujimura *et al*., 2012; Kimura 2004]. In our previous study, the tolerance of strain 06TSD19b to gastric acid was confirmed *in vitro* [Takeda, Ch. Tsend-Ayush *et al*., 2011].

The study found that the 06TSD19b strain is resistant to artificial gastric juice, as a result of which we believe that the 06TSD19b strain can survive in an acidic environment when it enters the stomach with food. Remarkably, strain 06TSD19b grows well in milk and fermented milk has a pleasant taste, improves the intestinal tract activity.

This was additionally confirmed in this *in vivo* experiment, in addition, it was found that cells of the 06TSD19b strain are able to attach to the stomach wall in mice infected with *Helicobacter pylori*.

Strain 06TSD19b inhibited the growth of *H. pylori* strain no. 130 and produced significant amounts of L-lactic acid, likely contributing to the anti-*H. pylori* activity *in vitro*.

Furthermore, strain 06TSD19b tended to adhere to gastric epithelial cells in *H. pylori* infected mice in this study, leading to a reduction in *H. pylori* colonization.

Thus, it was established that *L. paracasei*, isolated from traditional product, not only has a probiotic effect, also showed anti-*Helicobacter pylori* activity.

V.2 Клинические испытания молочного продукта с пробиотическими свойствами

For a fermented milk product with *Lactobacillus paracasei subsp. paracasei* (06TSD19b) was clinically tested. Clinical trials were conducted on healthy women as well as mice.

A double-blind crossover study designed to investigate the effects of ingesting fermented milk containing strain 06TSD19b was conducted.

Throughout the experimental period, the subjects regulated their diet to avoid eating other fermented products and products containing probiotics and oligosaccharides. Forty-six female students (18 − 39 years old) were randomly divided into 2 groups: those receiving fermented milk with probiotic or with nonprobiotic bacteria. Subjects participating in the experiment were forbidden to take other fermented milk products, as well as products containing probiotics and oligosaccharides. Observations were made for 1 week before the intake periods to obtain baseline values for the test. During the first intake period, the subjects

consumed 100 g of fermented milk twice a day (in the morning and evening) for 3 weeks. This was followed by a 1-week washout period, during which no fermented milk was consumed. Then, the subjects consumed the other fermented milk for another 3 weeks.

At the end of each week of the experiment, fecal samples were collected in sterile packs under anaerobic conditions.

During the fermented milk intake period, the L-lactic acid concentration tended to be higher in the group than the control group.

Ingestion fermented milk improved the subjects'fecal characteristics by increasing the L-lactic acid concentration in the intestine and modifying intestinal bacteria. Furthermore, RAPD analysis of Lactobacilli from the fecal samples indicated that fermented milk with probiotics can reach the intestine and remain viable.

CONCLUSION

Based on the results of the dissertation research, the following conclusions were obtained:

1. The study carried out on general chemical, amino acid, fractional composition, mineral and vitamin composition of goat, sheep cow's milk of pasture-raised local animals of Mongolia. Also, the study established that the milk of animals is of great value and is an affordable raw material for the production of fermented milk and with rich protein products for a preventive orientation.
2. Microorganisms and yeast have been isolated and identified. It has been established that the composition of the microflora of traditional fermented milk products tarag, airag, khoormog, byaslag include the following microorganisms: *Lactobacillus delbrueckii sub sp. bulgaricus, Streptococcus salivarius subsp. thermophilus, Lactobacillus helveticus, Lactobacillus fermentum, Lactococcus lactic ssp.lactis, Lactobacillus delbrueckii ssp. Lactis, Lactobacillus pentosus,Weissella confuse, Lactobacillus kefiri, Lactobacillus plantarum, Lactobacillus paracasei ssp.tolerans, Pediococcus parvulus, Lactobacillus paracasei ssp. paracasei, Leuconostoc mesenteroides,Weissella viridescens, Lactobacillus sakei, Lactobacillus pentosus, Lactobacillus buchneri, Leuconostoc citreum, Leuconostoc garlicum, Enterococcus durans, Enterococcus faecium, Leuconostoc argentinum, Leuconostoc Lactis, Bacillus lechiniformis* и *Brevibacillus invocatus*
3. The probiotic properties of lactic acid bacteria isolated from dairy products were studied. The isolated strains were tested for tolerance to low pH and bile acids, gas formation and adhesion on Caco-2 cells.
4. Microorganisms with a higher acid-forming ability were selected in order to obtain a starter culture. The selected microorganisms had the largest number of viable cells and were characterized by a more pleasant taste and aroma, had a delicate texture. Based on the results obtained, this microorganism can be recommended for use as starter cultures and probiotics in food biotechnology, in particular, in the dairy industry, for the production of functional foods. The fermentation technology for milk and dairy products has been developed.
5. *In vitro* and *in vivo* experiments have shown the effectiveness and prospects of using strains of lactic acid bacteria isolated from traditional dairy products to delevop technology of functional products with probiotic properties.

6. Technologies have been developed for fermented milk, protein and synbiotic functional products for children and for the population of different age groups.
7. Comprehensive studies have been carried out on the nutritional and biological value of the developed products. Regularities of changes in biochemical, microbiological, structural-mechanical and indicators of products during storage have been studied and as well as an expiration date has been set.
8. Clinical trials were indicated effect of fermented milk products, anti-*H. pylori* and therapeutic efficacy of the gastrointestinal tract.
9. Technical documentation was developed and pilot production of a fermented milk product with probiotic properties was permitted, which is confirmed the reproducibility of the developed technologies.

CONTRIBUTIONS

1. Lactic acid bacteria strains with high probiotic activity have been isolated that were based on 16S-ribosomal rDNA analysis and carbohydrate profile, they were identified as *Lactobacillus (L.) plantarum и L. paracase spp. paracasei.*

Original contribution to theoretical and applied sciences

2. Probiotic properties of lactic acid bacteria isolated strains were studied and as a result of the studies of the microflora of national dairy products, 10 homofermentative probiotic strains of lactic acid bacteria were found. The strains have been identified and classified as *L. plantarum, L. paracasei spp. paracasei*. It has been established that 6 out of 10 strains of lactic acid bacteria isolated from fermented camel's milk have probiotic activity.

Original contribution to theoretical and applied sciences

3. A collection of Mongolian microorganisms and the preparation of starter cultures that used in the production of fermented milk products has been created.

Original contribution to applied sciences

4. Clinical trials of fermented milk products have been carried out, anti-*Helicobacter* and therapeutic efficiency of the gastrointestinal tract has been recognized.

Original contribution to theoretical and applied sciences

5. It was found that *L. paracasei spp. paracasei (06TSD19b),* isolated from Mongolian traditional fermented milk products, not only has a probiotic effect, but also showed anti-*Helicobacter pylori* activity.

Original contribution to theoretical and applied sciences

6. For the first time in our country, a comprehensive study was conducted to characterize the properties of microorganisms isolated from Mongolian fermented milk products prepared using traditional technologies. An innovative technology for a number of fermented milk products with probiotic properties has been practically implemented in Mongolia and abroad.

Original contribution to theoretical and applied sciences

THE MAIN CONTENT OF THE DISSERTATION IS PUBLISHED IN THE FOLLOWING WORKS:

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2. Tsend-Ayush Ch., Functional food / Sh. Jadambaa, Ulaanbaatar, 2008. p.166.
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