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CHARACTERIZATION OF FUNCTIONAL AND MICROBIAL PROFILE OF WHEY RECOVERED FROM COTTAGE CHEESE AND CHEESE MANUFACTURING

Abbreviations

CFU- colony forming units; LAB – lactic acid bacteria; MRS – De Man, Rogosa and Sharpe agar; MPA – Meat Peptone Agar; BOD- biochemical oxygen demand; COD – chemical oxygen demand

The main reason for selecting whey as a substrate of research and raw material for alcohol production is to reduce industrial waste that adversely impacts the environment, as well as to make a profit on it. The environmental impact of whey is related to its biological oxygen demand (BOD = 230 mg/ml) and chemical oxygen demand (COD = 70 mg / ml). Whey is mainly composed of disaccharide lactose, and the activity of yeast strains plays an important role in its conversion to bioethanol. Lactic acid bacteria, whey composition, and fermentation conditions also play an important role. In this work, the physicochemical properties of milk and cheese whey were studied, and microbiological analysis was carried out. The results show the high quality of the whey of the two selected producers of cottage cheese and cheese. The uniformity of consistency and normal appearance, taste, and smell, corresponding to the whey, testify to the observance of all sanitary norms and rules at the stage of whey collection. No significant differences were found between the characteristics of two different whey samples. The cells of yeast strains isolated from whey had different natures. 4 strains of alcohol-resistant yeasts and 2 strain of lactic acid bacteria Lactobacillus plantarum W1 and Leuconostocmesenteroides W1 were isolated, which could be promising biocatalysts for bioethanol production.

Key words: milk whey, fermentation, yeasts, whey microflora.

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Ірімшік және сүзбе өндірісінде алынған сарысудың функционалды және микробтық профилінің сипаттамасы

Қысқартулар

ҚҚБ-колонияқұраушы бірліктер; СҚБ-сүт қышқылды бактериялар; MRS – агар Де Ман, Рогоза және Шарпа; ЕПА – ет-пептонды агар; ОБҚ – оттегіге биохимиялық қажеттілік; ОХҚ – оттегіге химиялық қажеттілік.

Этанол өндірісі үшін зерттеулер мен шикізат үшін субстрат ретінде сарысуды таңдаудың негізгі себебі қоршаған ортаға теріс әсер ететін өндірістік қалдықтардың азаюы, сонымен қатар пайда табу болып табылады. Сарысудың қоршаған ортаға әсері оның оттегіне биологиялық қажеттілігімен (ОБҚ= 230 мг / мл) және оттегінің химиялық қажеттілігімен (ОХҚ = 70 мг / мл) байланысты. Сарысу негізінен дисахаридті лактозадан тұрады, сондықтан ашытқы штамдарының белсенділігі оның биоэтанолға айналуына айтарлықтай әсер етеді. Сүт қышқылы бактериялары, сарысудың құрамы және ашыту шарттары да маңызды рөл атқарады. Бұл жұмыста сүзбе және ірімшік өндірісінен алынған сарысудың физика-химиялық қасиеттері зерттеліп, микробиологиялық талдау жүргізілді. Алынған нәтижелер таңдалған ірімшік пен сүзбе өндірушілердің сарысуының жоғары сапасын көрсетеді. Сарысуға сәйкес келетін консистенцияның біркелкілігі және қалыпты сыртқы түрі, дәмі мен иісі сарысуды жинау кезеңінде барлық санитарлық нормалар мен ережелердің сақталуын көрсетеді. Екі түрлі сарысу үлгілерінің сипаттамалары арасында айтарлықтай айырмашылықтар табылған жоқ. Са-

рысудан бөлініп алынған ашытқы штамдарының жасушалары әртүрлі сипатта болды. Спиртке төзімді ашытқылардың 4 штаммы және биоэтанол өндіру үшін перспективалы биокатализаторлар болуы мүмкін сүт қышқылы бактерияларының 2 Lactobacillus plantarum W1 және Leuconostocmesenteroides W1 штаммы бөлінді.

Түйінді сөздер: сүт сарысуы, ашыту, ашытқы, сарысу микрофлорасы.

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Характеристика функционального и микробного профиля сыворотки, полученной при производстве творога и сыра

Сокращения и обозначения

КОЕ – колониеобразующие единицы; МКБ – молочнокислые бактерии; MRS – агар Де Ман, Рогоза и Шарпа; МПА – Мясопептонный агар; БПК – биохимическая потребность в кислороде; ХПК – химическая потребность в кислороде.

Основная причина выбора сыворотки в качестве субстрата для исследований и сырья для производства этанола заключается в утилизации промышленных отходов, которые негативно влияют на окружающую среду, а также в получении наибольшей прибыли. Воздействие сыворотки на окружающую среду связано с ее биологической потребностью в кислороде (БПК = 230 мг/мл) и химической потребностью в кислороде (ХПК = 70 мг/мл). Сыворотка в основном состоит из дисахаридной лактозы, поэтому активность дрожжевых штаммов значительно влияет в ее превращении в биоэтанол. Молочнокислые бактерии, состав сыворотки и условия ферментации также играют важную роль. В данной работе были изучены физико-химические свойства творожной и подсырной сыворотки, проведен микробиологический анализ. Результаты показывают высокое качество сыворотки двух выбранных производителей творога и сыра. Равномерность консистенции и визуальные признаки, вкус и запах, соответствующие сыворотке, свидетельствуют о соблюдении всех санитарных норм и правил на этапе сбора сыворотки. Не было обнаружено значительных различий между характеристиками двух разных образцов сыворотки. Клетки дрожжевых штаммов, выделенных из сыворотки, имели разную природу. Выделено 4 штамма спиртоустойчивых дрожжей и 2 штамма молочнокислых бактерий Lactobacillus plantarum W1 и Leuconostocmesenteroides W1, которые могут быть перспективными биокатализаторами для производства биоэтанола.

Ключевые слова: молочная сыворотка, ферментация, дрожжи, микрофлора сыворотки.

1. Introduction

The rapid growth of industry, the development of cities and megalopolises, and the improvement of their amenities require solving problems associated with the negative impact of human activities on the environment. The food industry is one of the industries necessary to provide people with food. But at the same time, the food industry brings great harm to the environment. To reduce environmental damage, manufacturing is now beginning to use closed cycles, and in this new wave, the food industry has proven that the future is zero-waste. Secondary dairy raw materials are a good resource for the production of new economically significant products. Whey is the liquid that remains after the production of milk and dairy products such as cheese, cottage cheese and casein. At the moment, there are various ways of commercial uses of whey, as it is produced in large quantities and causes significant harm to the environment due to the content of many organic compounds in it. Cheese whey is the biochemical oxygen demand (BOD =230 mg/ml) and chemical oxygen demand (COD = 70 mg / ml) [1]. For the utilization of whey, more precisely for its chemical oxidation, about 50 g of oxygen is needed, in comparison, data on the oxidation of wastewater are given, for the oxidation of such waste 0.3 g of oxygen is required. It is for this reason that the whey has a deadly effect on the flora and fauna of the environment into which it will merge. Particular attention should be paid to Lactose, it is she who is largely responsible for the high level of BOD and COD. Isolation of protein and its use in the other direc-

tion reduces COD by only 12%. On the other hand, whey is very rich in nutrients such as peptides, minerals and vitamins, this side of it offers a promising possibility of using whey as a resource [2]. Whey is currently considered as a material for ethanol production based on the bioconversion of lactose.

Bioethanol is a renewable energy source without greenhouse gas emissions obtained by fermenting sugar-containing substances. Ethanol production is an excellent approach to energy independence and one of the best replacements for current polluting fuels such as fuel oil and coal obtained from traditional methods. today ethanol is important product on the fuel market [3]. Ethanol can be obtained in two ways, the first is obtained chemically, the second is enzymatic, using microorganisms that ferment sugars. At the beginning of the 20th century, the second method was used more often than the chemical method, but soon the choice of entrepreneurs changed due to the rise in prices for sugar and starch. In the chemical method for producing ethanol, reactions are carried out to hydrate ethylene; in the case of a fermentation or microbiological method, yeast is most often used to ferment sugars [4-6]. The production of ethanol using the fermentation process of sugar-rich materials is technically feasible, and its use does not affect the environment. In contrast to the production of ethanol using cellulosic materials, the use of whey is the most acceptable from a financial point of view, since cellulosic materials require additional manipulations, such as hydrolysis in order to break down into simple sugar molecules, which is expensive. Moreover, whey is very readily available for ethanol production [7].

Currently, most of bioethanol from secondary raw materials, in large-scale enterprises, is obtained using yeast. The type of yeast is of no small importance, since not all yeast can utilize lactose as the main sugar of whey. For example, *S. cerevisiae* yeast consume sugars such as glucose, fructose, maltose and maltriose, *S. diastaticus* – dextrins, and in turn *Klueyveromyces fragilis and K. lactis* consume lactose. Also used are genetically modified microorganisms such as *Zymomonas mobilis* and *Escherichia coli* for use in alcohol production. the use of such microorganisms doubles the efficiency of lactose fermentation [8].

The composition of whey, its microflora is of great importance in the production of ethanol and the isolation of the same strains from it for use in fermentation. Therefore, it is an important to understand and study the whey source prior to commencing industrial fermentation and whey distillation to obtain ethanol. The composition and properties of whey depends on many factors: the type of whey, milk pretreatment and processing parameters such as filtration, pasteurization, starter culture, rennet and salting will influence the whey composition [9, 10]. Minor compositional variations are likely to have little effect on the fermentation and distillation process; however, this can be a problem when striving to produce a clean, quality product with high yield. Criteria for the selection of microorganisms for ethanol production are also taken into account when creating a good technology. The tolerance of microorganisms to high temperatures and high ethanol concentrations are important characteristics for industrial use [11,12].

2. Materials and methods

Whey sampling

Two types of whey cottage cheese whey and cheese whey were used in the study. For this purpose, samples were taken from the LLP «Plant of the Kazakh Academy of Nutrition Amiran» dairy plant and the «Stella Alpina» cheese plant from the Almaty region. Samples were freshly collected and sterile packed. Organoleptic characteristics of the used whey were characterized.

Whey characterization

Fat content (FT), protein (PR), carbohydrates (CH) were evaluated using a scanner Lactoscan S. Additionally, the mass of dry matter, density (DE) and humidity by RADWAG MA 50.R Moisture Analyzer were pre-calibrated and validated, the mean study error is, 025% with determinations performed according to AOAC [13].

Determination of titratable acidity pH.

The method is based on titration of acidic salts, proteins, carbon dioxide and other components of whey samples with an alkali solution in the presence of phenolphthalein indicator. The titratable acidity is expressed in Turner degrees (° T). In a conical flask with a capacity of 150 cm3, we measured 10 cm3 of whey, and added three potassium phenolphthalein. The resulting mixture is thoroughly mixed and titrated with 0.1 N sodium (potassium) hydroxide solution until a stable faint pink color is obtained[14]. The volume of whey used for titration is multiplied by 10, since the titrated acidity in Turner's degrees is equal to the number of milliliters of 0.1 n alkali solution used to neutralize 100 cm3 of whey [15].

It is calculated according to the formula (1) No of ml. of 0.1 N NaOH solutions:

% Lactic acid = the amount of 0,1N NaOH used for titration / Weight of sample × 100

i.e. Weight of sample = Volume of whey × specific gravity)

Study of the microflora

To determine the microflora of whey samples and isolate pure cultures of yeast and lactic acid bacteria, the inoculation was carried out by the Koch method in two repetitions. Dilutions of 10^{-2}, 10^{-4} and 10^{-6} were selected. Inoculation was performed on solid culture media such as MRS, Sabourand Dextrose Agar and MPA. The cultivation lasted for two days (48 hours) at a temperature of 30 C. The average values of the number of colonies grown on Petri dishes of two seeding were taken. Identification based on physiological and biochemical characteristics of whey microorganisms were carried out on generally accepted in bacteriological practice methods using determinants [16-18].

Alcohol resistance test

To determine the resistance of yeast strains to the action of ethanol, nutrient media with the addition of ethyl alcohol were prepared until a concentration from 5 and 20 %. Collection strain *Klyuveromyces marxianus TD7* without alcohol addiction was used as a control. After incubation of the 8 samples for 72 hours, yeast colonies grew in place of the prints of the stamp-replicator. Alcohol resistance of the strains was evaluated by the number and size of the colonies [19].

Isolation of pure Ethanol Tolerant Yeast colonies and lactic acid bacteria.

Streak plate method. To obtain pure colonies of yeast and bacteria, the depletion streak method was used. Microorganisms were streaked into pre-prepared petri dishes with Sabouraud and MRS media. The cultures were incubated for two days at a temperature of 30˚C. Further, a quantitative and qualitative analysis was carried out, as well as microscopy. The finished cultures were transferred into a test tube with an agar medium and kept in a refrigerator until it was used in experiments.

Identification of LAB with Nucleotide Sequence Analysis

For sequencing, lactic acid bacteria were initially cultured in liquid MRS medium at 37 ° C for two days to accumulate cell culture. Lactobacilli were designated LAB1 and lactococci were designated LAB2.

Bacterial DNA was isolated using a special genetic kit PureLink® Genomic DNA Kits (Invitrogene, USA). Using a Qubitfluorimeter (Invitrogen, USA), the DNA concentration in samples was determined using the HS dsDNA scale.

Lactic acid bacteria were identified using special primers 8F (5'-AGAGTTTGATCCTGGCTCAG-3 ') and 806R (5'-GGACTACCAGGGGTATCTA-AT-3') based on the study of the 16S rRNA gene sequence [20]. The sample for identification consisted of a reaction liquid (30 µl) with the addition of 3 µl of 10-fold reaction buffer (Fermentas), 2.5 mM MgCl2, 0.2 mM of each deoxyribonucleotide (dNTP), 10 pmol of each primer, 1 unit of Taq polymerase Maxima Hot Start Taq DNA. Polymerase (Fermentas). The polymerase chain reaction was carried out in a MastercyclerproS thermal cycler (Eppendorf).

PCR analysis was started by incubating the mixture at 95 ° C for 7 minutes with thirty cycles, which consisted of: 95 ° C for 30 seconds, 55 ° C for 40 seconds, 72 ° C for 1 minute. The final extension of the nucleotide chain was carried out at 72 ° C for 10 minutes. Then the amplified initial product was separated in 1.2% agarosegel, stained with ethidium bromide and visualized in INFINITY VX2 gel (manufactured by VILBER LOURMAT, France). In the analysis, 1xTAE electrode buffer was used. The PureLink® PCR Purification Kit was used in the purification process.

DNA fragments of the 16S rRNA gene were sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit based on the manufacturer's protocol [21].

The end products of sequencing, after the action of the BidDye 3.1 terminator, were purified.

SeqA6 software was used to process the sequencing data. To search for similar nucleotide sequences of 16S rRNA genes, the BLAST database (Basic Local Alignment Search Tool) was used, and the search was also carried out in the Gene Bank International Database of the US National Center for Biotechnology Information [22]. The phylogenetic tree was constructed based on the Neiighbor-Joining (NJ) method.

3. Results and discussion

The data in Table 1 indicate the organoleptic characteristics of products of the selected two manufacturers of and cheese whey. Whey samples in terms of organoleptic indicators meet the requirements of the Customs Union [23].

Table 1- Organoleptic characteristics

Indicator name	Characteristics	
	Amiran (cottage cheese whey)	Stella Alpina (cheese whey)
Taste and smell	Pure milky taste and milky smell	Salty taste cheesy smell
Appearance and consistency	Homogeneous non-transparent liquid without precipitation	Homogeneous non-transparent liquid without precipitation
Color	Beige	Yellowish or pale green

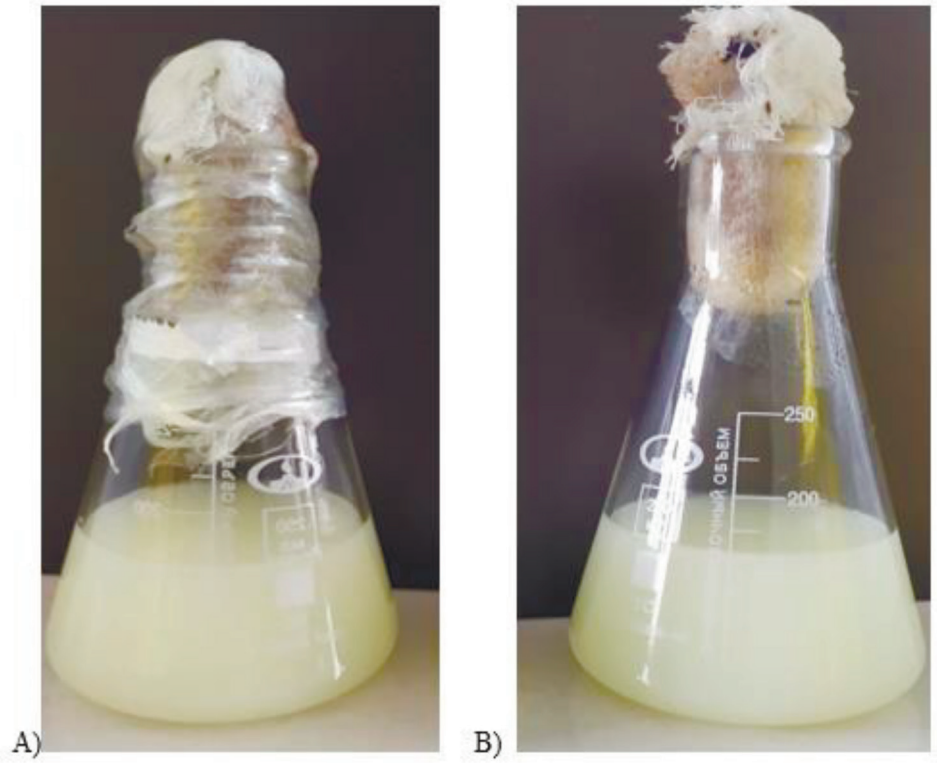


Figure 1 – Whey samples from two different manufacturers:A) LLP «Plant of the Kazakh Academy of Nutrition Amiran» dairy plant B) «Stella Alpina» cheese plant.

All indicators of physical and chemical characteristics of whey samples comply with the standards of the Customs Union [24]. According to the results (Table 2), it can be seen that the amount of sugars prevails in the composition of the whey, which plays an important role in the production of ethanol.

Table 2 – Physical and chemical indicators

Whey	Indicator						
	Fat %	Protein %	Carbohydrates %	Energy value, kilocalories	Moisture content %	Dry matter (g)	Density kg/m^3
Amiran (cottage cheese whey)	0.2	0,8	3,2	20 kcal., 83.6 kJ.	92.067	8	1018
Stella Alpina (cheese whey)	0.2	0,8	3,5	20 kcal., 83.6 kJ.	93.001	7	1022

Table 3 shows titratable acidity values at different pH levels. It was found that the initial titratable acidity in the whey from cottage cheese manufacture was 98 T˚ at pH 5, while the titratable acidity of cheese whey was 19 T˚ at pH 5.6. Within three days, the titratable acidity of the samples increased, while the pH decreased in both cases.

Table 3 – Titratable acidity and pH of whey

Whey type	Indicators	1st day	2nd day	3rd day
Cottage cheese whey	pH	5	4.8	4.6
	T˚	98	100	110
Cheese whey	pH	5.6	5.5	5.3
	T˚	19	21	23

Study of the microflora

Microbiological analysis of the obtained samples did not reveal the presence of extraneous microflora. The microflora of products is mainly represented by colonies of yeast, mold fungi, lactococci, as well as colonies of lactobacilli. On solid nutrient media of Sabouraud, most yeast cultures grew on Petri dishes as round, large colonies, the relief of colonies with a convex nipple-like white center and a roller along the periphery, the surface is shiny with a gloss, beige or white, pasty or curdled-granular consistency.

Colonies of lactococci grown on MRS medium were varied, mainly punctate colonies: small brown and white round colonies with transparent edges. There are colonies of lactobacilli – round, white, flat, shiny colonies with smooth edges of medium size. The number of live bacteria in 1 dose (0,05 ml) growing on MRS is 4.1x 105 CFU / mg, the number of live bacteria in 1 dose when growing on SDA is 5x 105 CFU / mg, the number of live bacteria in 1 dose when growing on MPA is 0, 8 x 105 CFU / mg.

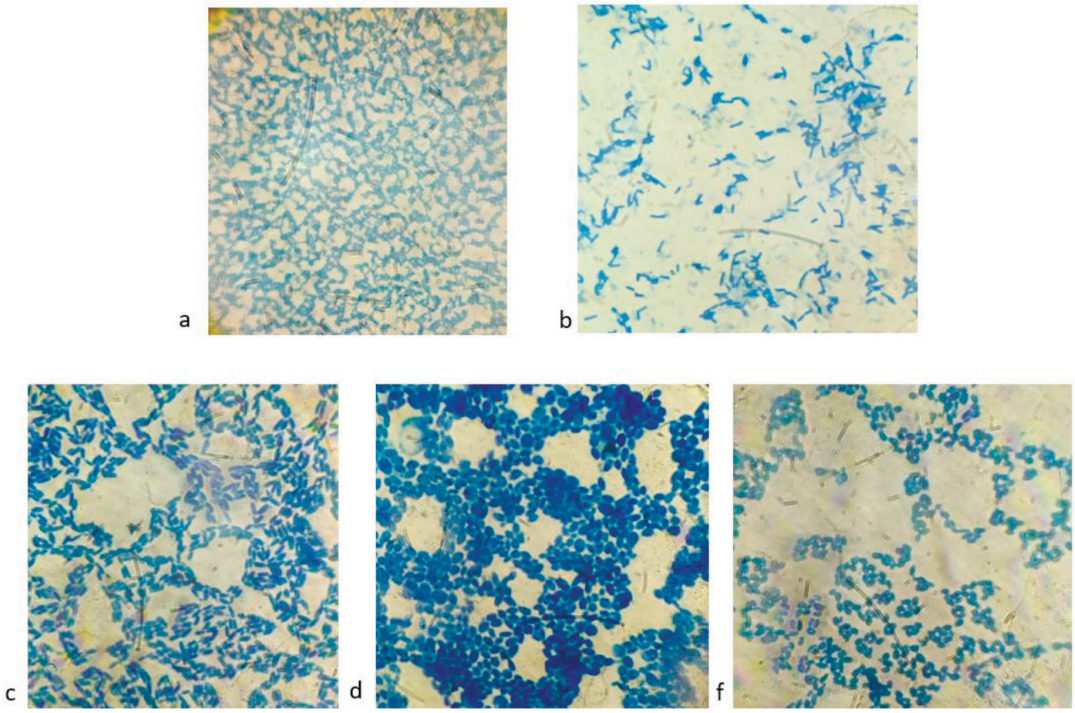


Figure 2 – The composition of microflora cottage cheese whey: Lactic acid bacteria (a, b); Yeasts (c, d and f)

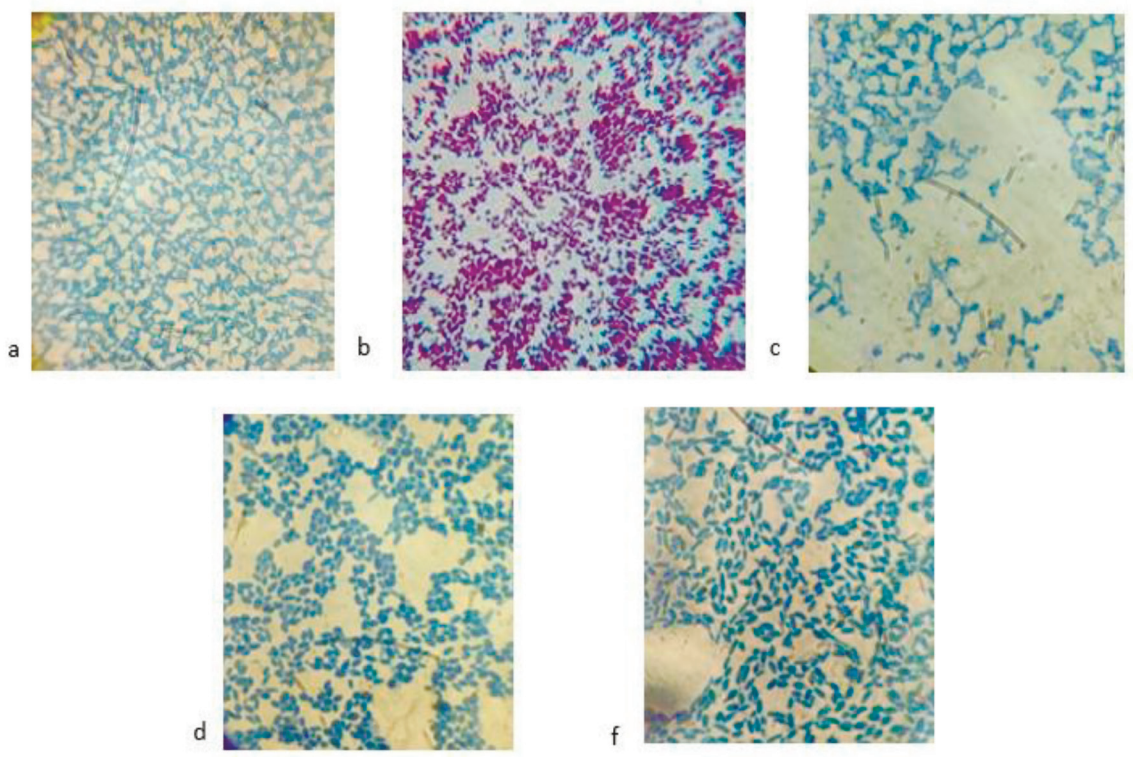


Figure 3 – The composition of the microflora of cheese whey (Stella Alpina): lactic acid bacteria – a, b) heat fixing b) gram staining; yeast colonies are d and f.

Basically, small colonies of lactic acid bacteria in the form of cocci grew on MRS media. Yeast of various forms grew on agar media of Sabouraud. Yeast cells have a cylindrical, ovoid, rounded, oblong shape with various sizes from 1.5 * 10 microns to 2.5-30 microns.

Ethanol Tolerance Test

The ability to be resistant to various environmental conditions such as ethanol resistance is one of the main criteria for selecting strains for an efficient ethanol yield. It is ethanol resistant strains that are used in the production of ethanol during fermentation, where the high resistance of the strains is very important. To determine the ethanol tolerance, the strains isolated from the whey were inoculated into TGY medium containing various concentrations of ethanol (5, 7, 9, 10, 12, 14, 16, and 20%).

Table 4 shows the concentration of ethanol added to the growth medium of various strains compared to *Kluveromyces marxianus TD7* as a reference. The results showed that all strains grew at an ethanol concentration of 5, 7, 9, 10%. Above this concentration, growth was observed only in some strains; ethanol acts on yeast cells and therefore we see suppression of yeast growth, decrease in cell volume, and high concentrations kill cells [25]. From different 8 strains of yeasts only 4 strains (Table 4) were tolerant to high ethanol concentrations.

Table 4 – Ethanol tolerance of the isolated strains.

Ethanol concentration (%)	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	*Kluveromyces marxianus TD7*
1	2	3	4	5	6	7	8	9	10
5	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+	+

1	2	3	4	5	6	7	8	9	10
9	+	+	+	+	+	+	+	+	+
10	+	+	+	+	+	+	+	+	+
12	-	+	+	+	+	+	+	+	+
14	-	+	-	+	+	+	-	+	+
16	-	-	-	+	+	+	-	+	+
20	-	-	-	+	-	-	-	+	+

Isolation of pure Ethanol Tolerant Yeast colonies and lactic acid bacteria.

1) Y4 – the yeast is cylindrical, elongated, about 1.5 * 12 μm in size – the colonies grew on petri dishes as round, large colonies (4-5 mm), the relief of the colonies with a convex nipple-like white center and a roller along the periphery, the surface is shiny with gloss, white, smooth edges, with a curdled-grainy consistency.

2) Y5- ovoid yeast, in the form of grains about 1.6 * 10 microns in size – the colonies grew on petri dishes as round, large colonies (4-5 mm), the relief of the colonies with a convex nipple-like white center and a roller along the periphery, the surface is shiny with glossy, white, with smooth edges, curdled-grainy consistency.

4) Y6- round yeast, about 1.8 * 18 μm in size – the colonies grew on petri dishes as round, medium colonies (3 mm), the relief of colonies with a convex top, the surface is matte, pale pink, with smooth edges, pasty consistency.

5) Y8-ovoid yeast, about 1.6 * 10 microns in size – the colonies grew on petri dishes as round, large colonies (4-5 mm), the relief of colonies with a convex nipple-like white center and a roller along the periphery, the surface is shiny with a gloss, white color, and curdled-grainy consistency.

6) Lactobacilli, rods 1.2 μm long. Colonies are small (1 mm), cream-colored with smooth edges, convex.

7) lactococci colony had medium size (1-2mm), bright white color, was smooth, convex and round shaped.

Yeast cells of the genus *Saccharomyces* of various shapes, usually round, oval or elliptical, while yeast cells of the genus *Schizosaccharomyces* are cylindrical with rounded ends

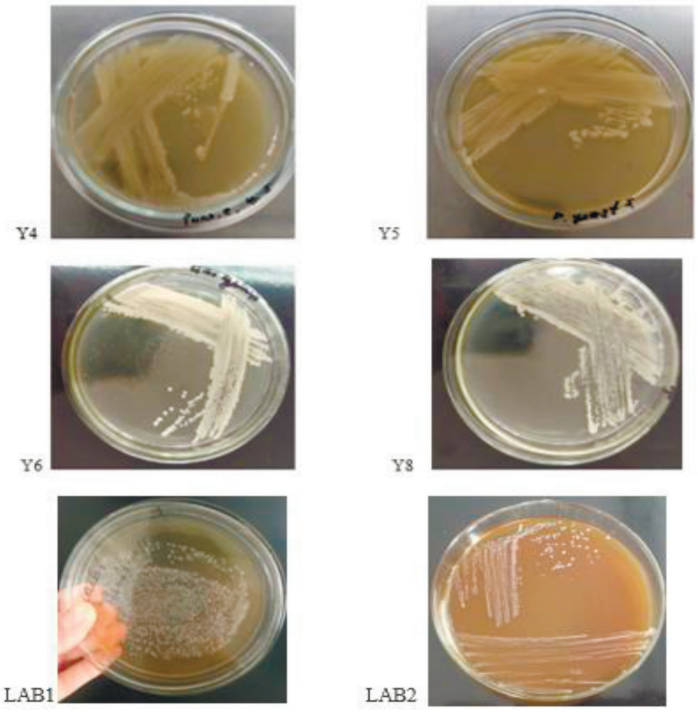
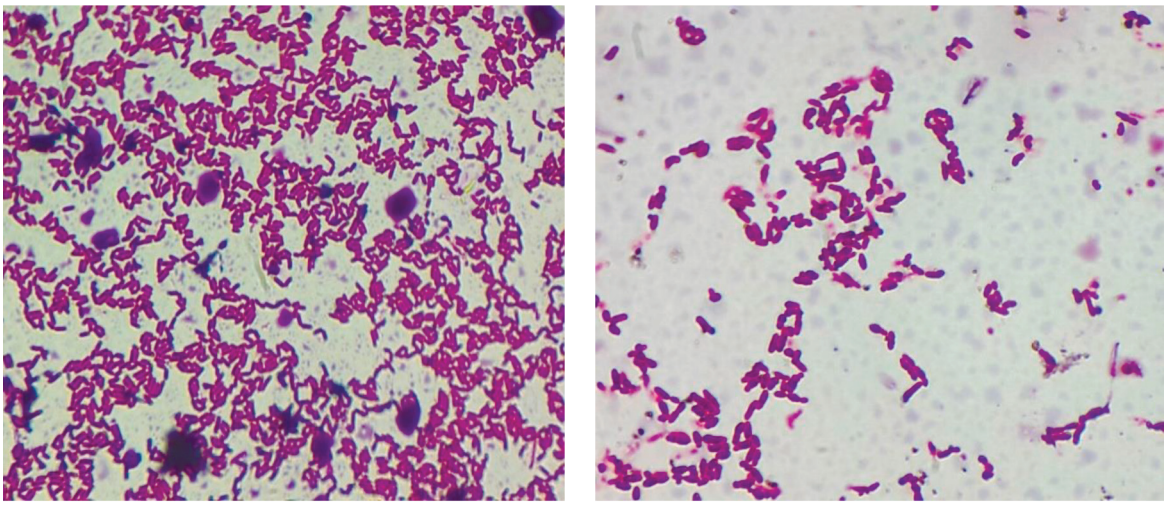


Figure 4 – Growth of colonies isolated from whey on SDA and MRS medium

Identification based on phenotypic, physiological and biochemical characteristics of cultures of yeasts and lactic acid bacteria were carried out according to generally accepted in microbiological practice methods using determinants [16-18]. Genotypic identification of LAB was performed with using amplification fragment of 16S rRNA gene [26].



a) LAB1 (bacilli) b) LAB2 (cocci)

Figure 5 – Microscopic images of chosen bacteria

Identification of LAB

Concentration of DNA in the samples were: sample # 1 – 12,8 ng/µI; # 2 – 2KG – 33,8 ng/µI.

After amplification with special primers for the 16S rRNA the PCR product with a size near 650 bp was obtained, as presented on the Figure 6.

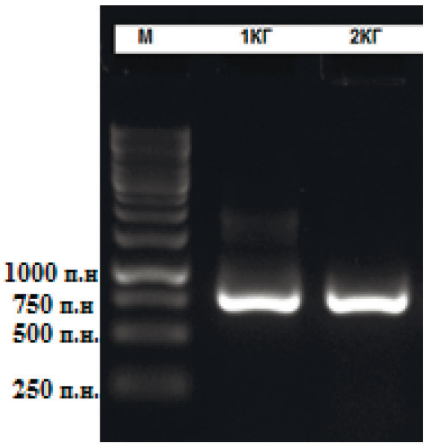


Figure 6 – PCR product obtained after amplification with universal primers

After the purification process sample contain PCR product #1 – 62.6 per ng/µI and in the sample #2 – 70,26ng/µI.

The data taken with the 3500 DNA Analyzer for capillary electrophoresis was processed using the SeqA6 software. Ultimately, the following nucleotide sequences were obtained:

The nucleotide sequence of the strain 1:

GAGTTGAGCTCCGGGCTTTCACATCAGACTTAATAAACCGTCTGCGCTCGCTTTACGCCCAATAAATCCGGATAACGCTCGGGACATACGTATTACCGCGGCTGCTGGCACGTATTTAGCCGTCCCTTTCTGGTATGGTACCGTCAAACTAAAATCATTTCCTATTCTAGCTGTTCTTCCCATACAACAGTGCTTTACGACCCGAAAGCCTTCATCACACACGCGGCGTTGCTCCATCAGGCTTTCGCCCATTGTGGAAGATTCCCTACTGCAGCCTCCCGTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGATCAGTCTCTCAACTCGGCTATGCATCATTGTCTTGGTAGGCCTTTACCCCACCAACTAACTAATGCACCGCGGATCCATCTCTAGGTGACGCCGAAACGCCTTTTAACTTTGTGTCATGCGACACTAAGTTTTATTCGGTATTAGCATCTGTTTCCAAATGTTATCCCCAGCCTTGAGGCAGGTTGTCCACGTGTTACTCACCCGTTCGCCACTCACTTGAAAGGTGCAAGCACCTTTCGCTGTGCGTTCGACTTGCAT

Alignment of nucleotide sequences was carried out to search for homologous nucleotide sequences of 16S rRNA genes using the BLAST program (Basic Local Alignment Search Tool) in the International Gene Bank database of the US National Center for Biotechnology Information, which showed that the strain under study belongs to the *Leuconostocmesenteroides* species (homology is 99%).) as shown in Figure 6.

The strain was named as *Leuconostoc mesenteroides W1.*

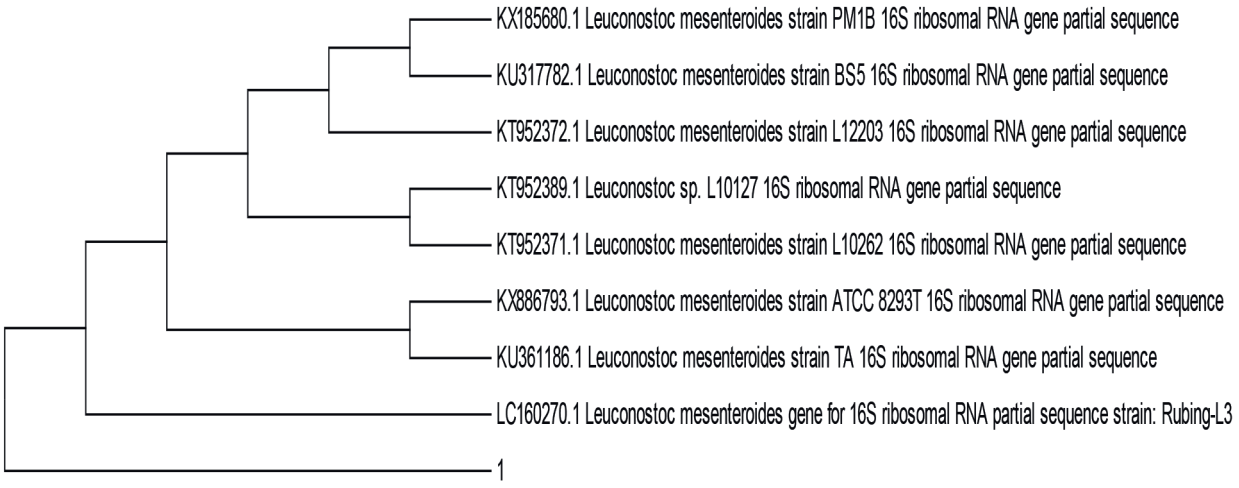


Figure 7 – Microbial cladogram of *Leuconostocmesenteroides W1*

Nucleotide sequence of the strain 2:

GGGGATAACACCTGGAAACAGATGCTAATACCGCATAACAACTTGGACCGCATGGTCCGAGTTTGAAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCGCGGCGTATTAGCTAGATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGCCGACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTAAAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTGGTAGTC

Alignment of nucleotide sequences was carried out to search for homologous nucleotide sequences of 16S rRNA genes using the BLAST program (Basic Local Alignment Search Tool) in the International Gene Bank database of the US National Center for Biotechnology

Information, which showed that the strain under study belongs to the *Lactobacillus plantarum* HUMBO7393.

This strain was named as *Lactobacillus plantarum W1*.

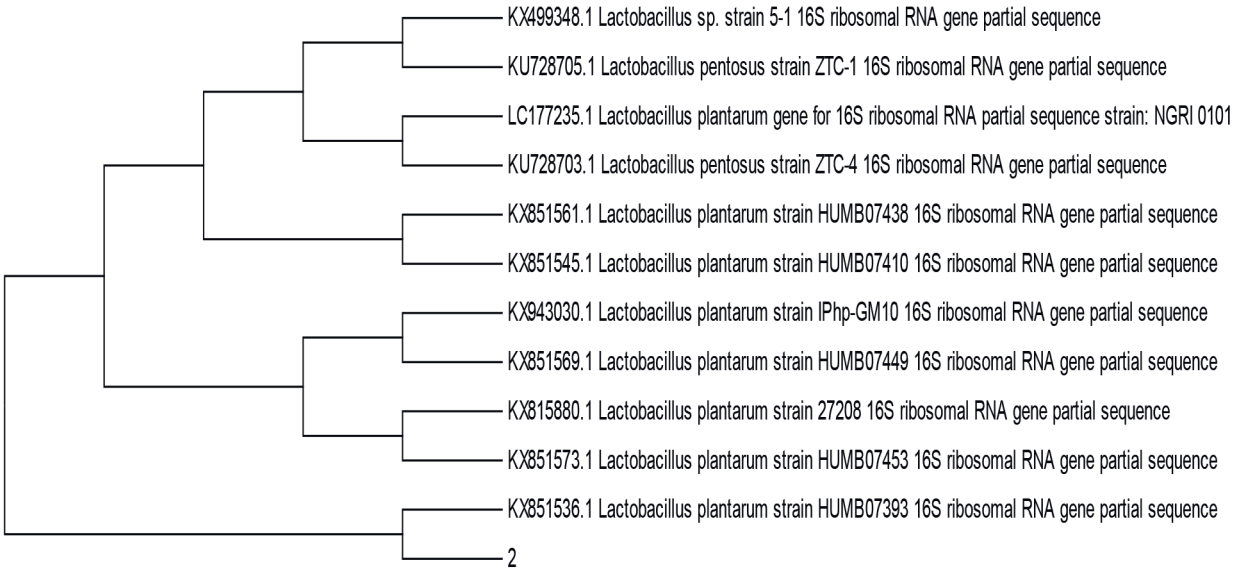


Figure 8 – Microbial cladogram of *Lactobacillus plantarum W1*.

Conclusion

The research has shown that uniformity of consistency and normal appearance, taste and smell corresponding to whey indicate compliance with all sanitary norms and rules at the stage of whey collection in the LLP «Plant of the Kazakh Academy of Nutrition Amiran» dairy plant and the «Stella Alpina» cheese plant. All physicochemical parameters of whey correspond to the required rules. It indicates that, through the process of fermentation, it will be possible to obtain a good quality ethanol in high yield. Moreover microbiological values within the referencenorms, can be the main moving force to bioconversion of lactose. Since the microflora of both whey is rich in yeast and lactic acid bacteria, which in tandem utilize lactose very well rather than separately. As a result, 4 different strains of yeasts Y4, Y5, Y6,Y8 were isolated, which are tolerant to the alcohol and 2 lactic acid bacteria strains *Lactobacillus plantarum W1* and *Leuconostocmesenteroides W1* which is perspective for further use in ethanol production.

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Conflict of interest

All authors have read and are familiar with the content of the article and have no conflicts of interest.

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