Fermentation conditions of lactobacilli for the production of lactose-free starter culture

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Abstract

A total of 16 strains of Lactobacilli were isolated from traditional Kazakh homemade dairy products. The strains identified by 16sRNA sequencing. The aim of this study was to evaluate probiotic properties of Lactic acid bacteria (LAB) Lactobacillus acidophilus (Lb. acidophilus) and Lactobacillus rhamnosus (Lb. rhamnosus) cultures were chosen as starter probiotic strains. Tested strains were had strong inhibitory effect against Gram-negative pathogens: Salmonella typhimurium (S. typhimurium), Serratia marcescens (S. marcescens) and Escherichia coli (E. coli). We evaluated the tolerance to various pH values (3.0, 4.0, 5.0, 6.4, 8.5), survivability in different salt concentrations (2%, 5%, 7%). All isolates survived at pH 3.0–4.0, 5–7% NaCl. The pasteurized milk (PM) was inoculated with two starters lactic acid bacteria performed for 24 h at 37°C. Lb. acidophilus and Lb. rhamnosus starter culture reached cell population of about ~ 8 logs colony forming unit (CFU/mL) during co-culture fermentation, pH in pasteurized milk rapidly decreased to 3.0.

Introduction

Lactose malabsorption is a widespread condition in the world because of is caused by a decline in lactase activity. Lactose, sometimes called milk sugar. Normally, the lactase enzyme hydrolyzed lactose into its monosaccharides components, glucose and galactose (Michele et al. 2002; Frederick et al. 2010; Nevin and Murray 1988).

The symptoms of lactose intolerance is a chronic gastrointestinal disorder characterized by abnormal pain, diarrhea, bloating, weight lost, flatulence and abdominal cramps (Claudia et al, 2020; Alicja et al. 2020). Lactose intolerance affected about 75% of the world population and may be connected with different genetic factors. The global prevalence of lactose intolerance is 2–5% in Northern Europe (Scandinavia, Germany, Great Britain), 17% in Finland and Northern France, about 50% in South America and Africa. In North American adults (79% of Indigenous Americans, 75% of African-Americans, 51% of Hispanics, and 21% of Caucasians). And between 90 and 100% in Southeast Asia including the Republic of Kazakhstan (Ralf et al. 2017). Furthermore, many studies have shown that avoidance of milk and dairy products can increase the risk of bone fracture, osteoporosis and nutrient deficiencies. However, milk products are the main source of calcium, potassium, vitamin D, B vitamins and high-quality protein (Rosaura et al. 2020).

Parisa et al. (2017) reported that bacteria, yeast, and fungi may be used as probiotics. Especially the LAB selected as probiotics cultures. Several other researchers reported that starter culture bacteria (Lb. acidophilus, Lb. plantarum, Lb. rhamnosus, Bifidobacterium breve, B. lactis, B. longum and Streptococcus thermophiles) is the activity of B-galactosidase, which is naturally reduced the lactose thus making the product suitable for lactose-intolerant people (Peera and James 2013; Zaheer et al. 2013). Probiotic foods must contain living microorganisms. The connection of different microbial populations belonging to the Lactobacillus has been used traditionally in fermented dairy products as probiotics (Michael and Schrezenmeir 2008).
In 1970s the idea of probiotics first was founded by the Russian-born Nobel Prize winner Elie Metchnikoff, known in the book «The prolongation of life». The term probiotics first introduced by W.Kollath as «the microbial population favorable to the gut microflora». Then, Fuller listed the following as criteria of a good probiotics. Later, Fuller suggested the definition as «Preparation of live microorganisms that is consumed by humans and inducing beneficial effects by qualitatively or quantitatively influencing their gut microflora». After that, Floch MH, Madsen KK and Jenkins DJ broadened the definition to «Probiotics are human-associated microorganisms which are consumed either with food or as a supplement with the purpose of improving the health of the host». The last update on the definition of probiotics was in 2018 by the United States National Institutes of Health: National Center for Complementary and Integrative Health. The redefined as «Probiotics are viable organism is intended to provide a health benefit when consumed, generally by improving the gut flora. Products sold are probiotics include dairy foods and products aren’t used orally, such as skin creams» (Parisa et al. 2017; Michael and Schrezenmeir 2008).

Nadia et al (2016) reported that probiotic fermented products should contain at least $10^7$ CFU/g of this alive bacteria. Nowadays, probiotics have been shown to improve lactose metabolism. Selection criteria for the probiotics include being of decrease pH and lactose and an increase lactic acid concentration. Having biological activity against pathogen and colonizing the gastrointestinal tract (Phebe et al. 2015).

The aims of this work were to determine the viability of simulated gastrointestinal transit and antagonistic research potential probiotic starter bacteria *Lb. acidophilus, Lb. rhamnosus*. Identify the fermentation period to reach low pH values of PM during mixed culture by *Lb. acidophilus* and *Lb. rhamnosus*.

**Material And Methods**

**Isolation and enumeration**

The 16 LAB strains were isolated from hurt fermented cow milk products (kurt, irimshik), liquid fermented cow's milk products (ayran, homemade butter), fermented horse's milk products (kumis) and camel's milk (shubat). Twelve samples were collected from a suburb called Zhibek Zholy, located in the Arshaly district, Akmola region, in June 2020. About 100 ml of each sample was collected and stored in a sterile glass bottle. Then samples were transported to our laboratory, further was made microbiological analysis of LAB and isolation.

Hurt objects like «kurt» and «irimshik» was rubbed with porcelain mortar and pestle. Then samples were moistened with physiological saline NaCL. Cycloheximide at a concentration of 0.01 was added to the MRS to inhibit the growth of fungi. The isolation of bacteria was carried out according to Abosereh et al. (2016) with some modification.

Serially diluted samples (one milliliter from each sample starting from $10^7$, $10^8$, $10^9$) were examined on MRS agar. Plates were incubated under anaerobic conditions at 37 °C for 48 h. Lactobacilli colonies have
been randomly chosen and enumerated. To obtain well separated purified colonies, this process was repeated three times. Then, the strains were phenotypically researched by morphology and Gramm staining.

**Identification and phylogenetic analysis**

The identification of strains was performing using the 16S rRNA gene was amplified by PCR using the forward primer 8f 5’ – AgAgTTTgATCCTggCTCAg-3 and 806R- 5’ ggACTACCAgggTATCTAAT. PCR amplification was including long denaturation at 92 °C for 3 minutes; 32 cycle: 95 °C - 3 s, 55 °C – 40 s, 72 °C – 60 s; nal elongation 10 minute at 72 °C.

The PCR program was performed using a GeneAmp PCR System 9700 thermocycler (Applied Biosystems). The PCR products were purified from unbound primers by the enzymatic method using Exonuclease I (Fermentas) and alkaline phosphatase (Shrimp Alkaline Phosphatase, Fermentas). The sequencing reaction was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applide Biosystems) according to manufacturer’s instructions, followed by separation of the fragments on an automatic analyzer 3730xl DNA Analyzer (Applide Biosystems). The resulting sequences were identified in GeneBank using the BLAST algorithm. The Mega version 6.0 software was used to create phylogenetic trees by the Neiighbor –Joining (N-J) algorithm (Dan et al. 2016).

**Antimicrobial activity**

Antimicrobial activity of *Lb. acidophilus* and *Lb. rhamnosus* was evaluated by agar diffusion (agar spot test) methods as described by Rain et al. (2019). The indicator strains used in this study were *S. typhimurium*, *S. marcescens* and *E. coli*. The inhibitor tests were performed in triplicate.

Lactobacilli were inoculated in MRS broth at 37 °C for 24 h under anaerobic condition. One hundred microliters of the targeted pathogens 10⁷ CFU/ml were poured plated on MRS agar and left 4 h to solidify in the refrigerator to avoid early growth of the strain. Plates were incubated at 37 °C for 24 h. Antagonistic activity of the probiotic strains were recorded of the formation inhibition zones (mm) around the wells. High zone of inhibition – 10 – 5.5 mm, middle zone of inhibition– 5.0 - 8.9 mm, low zone of inhibition – 1.0 - 4.9.

**pH treatment and viability**

The tolerance to pH was verified according to methodology described by Laenia et al. (2020) with some modification. One milliliter of the probiotic strains were added with 9 mL sterile distilled water, to take an initial dilution. Serial dilutions were made separately for *Lb. acidophilus* and *Lb. rhamnosus*. The initial pH values of the MRS medium were adjusted to 3.0, 4.0, 5.0, 6.4 and 8.5 with 1 mol/L HCL and 1 mol/L NaOH. The pH was measured using a «Mettler Toledo SevanCompact». Tested probiotic culture incubated at 37 °C for 48 h, 72 h and 120 h. All cell concentrations were representing as log CFU/mL.
The number of CFU was calculated by the formula:

\[ M = \frac{a \times 10^n}{V} \]

M – the number of cells in 1 ml; a – the average number of colonies grew from dilution; V – the volume of the suspension ml; \(10^n\) – the dilution.

**NaCL treatment and viability**

The ability of LAB to survive under different salt concentrations was studied in MRS broth and agar. The tolerance to NaCL was determined according methods by Claudia et. al (2014). One milliliter of samples was mixed with 9 mL distilled water to take an initial dilution. Serial dilutions were made separately for *Lb. acidophilus* and *Lb. rhamnosus*. Thus, 1 mL suitable dilution *Lb. acidophilus* and *Lb. rhamnosus* were mixed with the MRS broth with different additions of NaCl (2%, 5%, 7%) and inoculated at 37 °C for 48, 72 and 120 h. Viable counts of probiotic bacteria were obtained by spreading 1 mL of the appropriate dilution were mixed with the melted MRS agar. The MRS plates containing 0.1% (w/v) cysteine-HCl allowing differential count, the colonies presented of color white. Plates were incubated under anaerobic condition at 37 °C for 48 h. Then colonies enumeration was expressed as log CFU/ml.

The number of CFU was calculated by the formula:

\[ M = \frac{a \times 10^n}{V} \]

M – the number of cells in 1 ml; a – the average number of colonies grew from dilution; V – the volume of the suspension ml; \(10^n\) – the dilution.

**Statistical analysis**

Significant differences of means (\(p \leq 0.05\)) were compared through independent Student’s t-test by using SPSS 23 (IBM Corp., Armonk, New York, USA). Statistical analyses were carried out using Microsoft Excel 2010.

**Results**

**Isolation and enumeration**

We selected seventeen samples of bacteria from six traditional Kazakh homemade dairy products. Their colonies (2 – 6 mm diameter) had an oval or round shape, smooth surface, plat profile, shiny and white color appearance on MRS agar. Identification of Lactobacilli isolates was carried out by examination of colony characteristics and cell morphology.
The isolated LAB strains was identify with 16S RNA. The 16S RNA sequence data were aligned to conduct a phylogenetic tree and the phylogenetic position of studied strains. The sequence of the 16S rRNA gene (around 1,400 bp) was determined and searched with the NCBI BLAST program (http://www.ncbi.nlm.nih.gov) for their closest relatives/reference strains with a homology of over or equal to 99%. In the process of genetic identification, it was found that the studied cultures belong to taxonomic group such as Lactobacillus (fig. 1).

The phylogenetic trees showing were Lactobacilli (Lb.) containing (Lb. fermentum, Lb. gorillae, Lb. durianis, Lb. agilis, Lb. equi, Lb. alimentarius, Lb. zymae, Lb. saniviri, Lb.rhamnosus Lb.paracasei, Lb. zeae, Lb. algidus, Lb.dextrinus, Lb. acidophilus, Lb. equicursoris).

Previously we have studied probiotic properties of all the Lactobacilli isolates: antagonistic activity against pathogenic microorganisms, biocompatibility, viability and lactose utilization activity (Raikhan et al. 2020). Further research will focus on Lb. acidophilus and Lb.rhamnosus. The ability of Lb. acidophilus and Lb.rhamnosus to ferment lactose had been investigated using Cole’s ferricyanide methods (Sydney 1933). The lactose content of the PM with cultivated Lb. acidophilus and Lb.rhamnosus was 3.1 % (data not shown). In the traditional Kazakh homemade dairy products Lb. acidophilus and Lb.rhamnosus was identified as dominant species and present in the most collected samples.

**Antagonistic activity**

The antagonistic activity of the sixteen tested probiotic strains was studied by agar spot test. The antagonistic activity of LAB against three indicator pathogens widely differ, the results are showed in table 1. The Lb. acidophilus and Lb. rhamnosus possessed the high and middle zone of inhibition effect against all 3 indicator pathogens S. typhimurium, S.marcescens and E.coli (table 2).

**Table 1** Antagonistic activity of 16 strain of LAB isolated from traditional Kazakh homemade dairy products
<table>
<thead>
<tr>
<th>Strains</th>
<th>Identifications</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lb 1</td>
<td><em>Lb. fermentum</em></td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Lb 2</td>
<td><em>Lb. gorillae</em></td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Lb 3</td>
<td><em>Lb. durianis</em></td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Lb 4</td>
<td><em>Lb. agilis</em></td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lb 5</td>
<td><em>Lb. equi</em></td>
<td>-</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Lb 6</td>
<td><em>Lb. alimentarius</em></td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Lb 7</td>
<td><em>Lb. zymae</em></td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Lb 8</td>
<td><em>Lb. saniviri</em></td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Lb 9</td>
<td><em>Lb. rhamnosus</em></td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Lb 10</td>
<td><em>Lb. paracasei paracasei</em></td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lb 11</td>
<td><em>Lb. paracasei tolerans</em></td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lb 12</td>
<td><em>Lb. zeae</em></td>
<td>+</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Lb 13</td>
<td><em>Lb. algidus</em></td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Lb 14</td>
<td><em>Lb. dextrinicus</em></td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lb 15</td>
<td><em>Lb. acidophilus</em></td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Lb 16</td>
<td><em>Lb. equicursoris</em></td>
<td>++</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

+: low zone of inhibition (1.0-4.9 mm), ++: middle zone of inhibition (5.0-8.9 mm), +++: high zone of inhibition (10 – 5.5 mm), -: no inhibition. Indicators, I: *E. coli*, II: *S. typhimurium*, III: *S. marcescens*

**Table 2** Antagonistic activity of potential probiotic cultures against Gram-negative pathogen

The inhibition high zone range was exhibited by *Lb. acidophilus* against *E. coli* and *S. marcescens* from 10-12 mm, respectively. While, obtained middle inhibition zone against *S. typhimurium* was 6.0 mm. *Lb. rhamnosus* of also was effective against all indicator bacteria. The *Lb. rhamnosus* showed high zone inhibition against *E.coli* and *S. marcescens* were 10 mm. A meddle zone of inhibition was showed against *S. typhimurium* 5.0 mm, respectively.

**pH treatment and viability**

The pH effect on the growth properties of potential probiotic culture *Lb. acidophilus* and *Lb. rhamnosus* was carried out as described in the material and methods section. The ability of potential probiotic
strains to survive in acid conditions is a required criterion for the production of probiotic dairy products (Abosereh et al. 2016).

It is suggested that a good probiotic must have the ability to withstand a pH of range 3.5 – 4.5 because it is used to evaluate the acid tolerance of a probiotic culture (Jung et al., 2016).

As pH is an important criterion for the functioning and viability of bacteria, we research the effect of various pH values (3.0, 4.0, 5.0, 6.4, 8.5). The evolution of cell populations during cultivation of \textit{Lb. acidophilus} and \textit{Lb. rhamnosus} are shown in fig. 2, fig. 3.

\textit{Lb. acidophilus} and \textit{Lb. rhamnosus} shows a good growth at pH 3.0. The cell populations remained $\geq 10^6$ CFU ml during 72 h incubation. When \textit{Lb. acidophilus} was treated of pH values 3.0, 4.0, 5.0 the cells population was significantly higher than that of the pH 6.4, 8.5 treatment group ($p \leq 0.05$).

As, shown in fig. 2, fig. 3 the \textit{Lb. acidophilus} had been reached the population from 6, 38 to 6, 52 log CFU. \textit{Lb. rhamnosus} from 6, 35 to 6, 38 log CFU, during fermentation from 24 to 72 h of pH 4.0, it increasing was statistically significant ($p \leq 0, 05$). A rapid cells raised in the pH 3.0 was observed by \textit{Lb. acidophilus}, reaching from 6, 67 to 6, 72 at 72 h of fermentation ($p \leq 0, 05$). However, at pH 4.0 from 48 to 72 h and pH 5.0 from 48 to 72 h the cell population decreased slightly, but it was not statistically significant ($p \geq 0,05$).

\textit{Lb. acidophilus} and \textit{Lb. rhamnosus} doesn’t grow well above 5.0. The viable cell counts decline rapidly in the range of pH 6.4 – 8.5. At the end of the study, \textit{Lb. acidophilus} decreased by $\sim 1.4 \text{ log CFU}$, \textit{Lb. rhamnosus} $\sim 1.6 \text{ log CFU}$, respectively from the initial counts.

**NaCL treatment and viability**

To perform a selection of possible probiotic candidates we investigate their ability to grow in various salt concentrations. Salt tolerance of probiotic strains is one of the important technological properties for the production of probiotic dairy products. The osmotic stress may cause pronounced inhibition for bacterial growth. Therefore, that a high concentration of NaCL caused a decline in the adhesion ability of functional groups of lactobacilli and provides increases in the cell membrane damage (Jiage et al. 2020).

Thus, \textit{Lb. acidophilus} and \textit{Lb. rhamnosus} were tested for their ability grow under different salt concentration (2\%, 5\%, 7\% NaCL). Results of the cells growth are presented in fig. 4, fig. 5. The \textit{Lb. acidophilus} and \textit{Lb. rhamnosus} showed not significantly increases of the cell population during 24, 48 and 72 hours under 2 \% NaCL treatment, which do not differ statistically ($p \geq 0.05$). The number of the cells in medium containing 5\% NaCL was significantly higher than that in 2 \% NaCL ($p \leq 0.05$). The tested strains show the best survival rates in 5\% and 7\% tolerance to NaCl. \textit{Lb. acidophilus} and \textit{Lb. rhamnosus} were found to grow at $\sim 7 \text{ log CFU}$ at 5 – 7 \% NaCl during 72 h.

**Co-fermentation of \textit{Lb. acidophilus} and \textit{Lb. rhamnosus}**
The counts of *Lb. acidophilus* and *Lb. rhamnosus* strains during co-fermentation are shown in Fig. 6. At the beginning of co-fermentation ~ 7 log CFU/ml of *Lb. acidophilus* and *Lb. rhamnosus* were inoculate into 500 ml PM. Co-culture fermentation process was carried out 24 h. The viable cell populations of tested strains increased slowly over 24 h and reached ~8 log CFU/ml, which showed an increase of 1 log CFU/ml. Within 8 hours of co-fermentation, their growth was above 7 log CFU/ml. *Lb. rhamnosus* population ranged from ~ 7.29 to 7.57 log CFU/ml at 20 h. *Lb. acidophilus* showed a population was 8 log CFU/ml at the end of co-cultured fermentation. In the co-culture of *Lb. acidophilus* and *Lb. rhamnosus* both presented increasing their population from ~ 7 log CFU/ml to ~ 8 log CFU/ml in 12, 16, 20 and 24 h of fermentation than when cultivated in 4 h of fermentation, this differences was significant (p ≤ 0.05). Thus, the LAB reached significantly viable counts of cell population in 24 h of fermentation, than when cultivated in 12, 16, and 20 h (p ≤ 0.05).

The acidification rate during co-fermentation of mixed culture in PM is showed in fig.7. At the 4 hours, the pH values fall in the PM substrate with inoculum. Co-cultured fermentation with *Lb. acidophilus* and *Lb. rhamnosus* followed decrease pH from 6 to 3 at 24 hours.

**Discussion**

Probiotic bacteria should be able to survive and pass through the gastrointestinal tract (Jung et al. 2019). Therefore, this study was carried out to evaluate the several probiotic characteristics of *Lb. acidophilus* and *Lb. rhamnosus*.

Three common pathogens *S. typhimurium, S.marcescens and E.coli*, were used for the examination of antagonistic activity, carried out in vivo diffusion methods. All of the two strains have shown strong antimicrobial activity against *E.coli*. However, *Lb. acidophilus* the inhibition was more pronounced than *Lb. rhamnosus* (Table 2).

As demonstrated by Yongtao et al. (2018) *Lb. acidophilus* inhibited not only the growth of Gram-negative bacteria but also the growth of Gram-positive bacteria like *L. monocytogenes, S. aureus* and *S. hemolyticus*. Similar observations were made by Augustine et al. (2016) that *Lb. rhamnosus* could inhibit several pathogens. Muhammad et al. (2017) has reported that *Lb. rhamnosus* was higher inhibition zone against *E. coly* (with a diameter 8 mm – 13 mm).

*Lb. acidophilus* and *Lb. rhamnosus* are bacterium that produced lactic acid. The bactericidal activity of *Lb. acidophilus* towards gram-negative pathogen could be associated with the production lactic acid from the glycolysis of carbohydrates (Ralitsa et al. 2015; Claudia et al. 2014).

Our studies have shown that *Lb. acidophilus* and *Lb. rhamnosus* have the broadest activity spectrum and zone inhibitions (with high and middle diameter range between 5 mm– 12 mm).

These strains were further examined for resistance to the pH and NaCL, for the ability to grow at different acid conditions and salt concentrations.
Kaila and James (2000) in their work reported that pH values ranging from 3.5–4.5 are a good environmental condition for populations of the potential probiotic organisms. Survival of *Lb. acidophilus* and *Lb. rhamnosus* were based on pH value; low pH increases their survival. Figure 2, 3 shows survival rates of the *Lb. acidophilus* and *Lb. rhamnosus* at 3.0, 4.0, 5.0, 6.4 and 8.5 during 72 h incubation. At pH 3.0–4.0, the survival rate was higher compared to at pH 6.4–8.5 for all tested strains (p ≤ 0.05). This result showed that all research strains have high resistance to acid conditions. Phebe et al. (2015); Sorbhi et al. (2012) have studied survival of *Lb. rhamnosus* and *Lb. acidophilus* in acidic conditions showed greatest survival (pH 1.5–3.0, pH 3.0–4.0). Federico, et al. (2014) reported that the experiment revealed that acid condition plays an important role for bacterial growth.

Tolerances to acidity in the pH range 3.0 – 4.0 suitable for probiotic cultures are sufficient to successfully pass through the stomach and the surrounding of the human gastrointestinal tract. These results match with most data reported by other author (Emine and Merih 2021; Nadia et al.2016).

*Lb. acidophilus* and *Lb. rhamnosus* were tested for their abilities to grow in the presence of NaCL (2%, 5%, 7%). Two strains were tolerant against NaCL concentrations. The *Lb. acidophilus* and *Lb. rhamnosus* reached ~ 6 Log CFU/ml, respectively in 48 h.

Other researchers have also observed high rates of bacteria at 7 – 5% NaCL, that *Lb. acidophilus* strain was increased by 7 – 5% of NaCl among the probiotic strains used in this study (Vinderola et al. 2002; Juan et al. 2019). Thus, 7 – 5% NaCL stimulated growth of the strains.

NaCL is widely used in food manufacturing, but in probiotic products, the high salt concentration influences the cell viability, metabolism, and physiological functions of the isolates (Jiage et al. 2020).

It has been suggested that probiotic products required bacterial suspension (1x10^7 CFU/ml) in order to give health effects to the digestive systems (Sung-Ho et al. 2017). The co-fermentation process for the preparation of fermented probiotic foods are depicted in Fig. 6. The highest level of *Lb. acidophilus* and *Lb. rhamnosus* observed this required dose at the level above ~ 8 logs CFU/ml at the end of co-cultured fermentation (Fig. 6). The counts of probiotic bacteria observed are in agreement with similar studied conducted by previous authors (Katarzyna et al. 2019).

**Conclusion**

The present study describes antagonistic activity, tolerances (acid and salt concentrations) of *Lb. acidophilus* and *Lb. rhamnosus* strains isolated from traditional Kazakh homemade dairy products. Studied culture possessed significant degree of resistance against low pH and 5–7% NaCL. Based on these results, it can be conducted that *Lb. acidophilus* and *Lb. rhamnosus* are probiotic isolates and might be used as a starter for dairy fermented foods. A cell population of *Lb. acidophilus* and *Lb. rhamnosus* at ~ 8 logs CFU/mL and a pH value 3.0 was achieved within 24 h of co-cultured fermentation process.
Declarations

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Competing interests

Author Masalimov Zhaksylyk Kairbekovich, Sagyndykov Utemurat Zulhanaevich declares they have no financial interest.

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Non-financial interests: Masalimov Zhaksylyk Kairbekovich, Sagyndykov Utemurat Zulhanaevich have served on advisory boards for PhD student Imanbayeva Madina Kairtaevna.

Author contributions

Material preparation, data collection and analysis were performed by Imanbayeva Madina Kairtaevna, Masalimov Zhaksylyk Kairbekovich, Sagyndykov Utemurat Zulhanaevich. The first draft of the manuscript was written by Imanbayeva Madina Kairtaevna and all authors commented on previous version of the manuscript.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material
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**Figures**
Figure 1

Phylogenetic tree based on 16S rRNA sequences showing the positions of Lb. acidophilus and Lb. rhamnosus
Figure 2

Changes in viable cell counts (differences between counts at 24, 48, 72 h, in log CFU ml of \textit{Lb. acidophilus} at different pH values. Bars indicate standard deviation of the mean. Indicates significant different (p ≤ 0.05) using the Student’s \textit{t} test. Different upper-case letters indicate significant differences between cell populations within the different indication time within the same stress treatment.
Figure 3

Changes in viable cell counts (differences between counts at 24, 48, 72 h, in log CFU ml of *Lb. rhamnosus* at different pH values. Bars indicate standard deviation of the mean. Indicates significant different (*p* ≤ 0.05) using the Student’s *t* test. Different upper-case letters indicate significant differences between cell populations within the different indication time within the same stress treatment.
Figure 4

Changes in viable cell counts (differences between counts at 24, 48, 72 h, in log CFU ml of *Lb. acidophilus* at different NaCl concentration. Bars indicate standard deviation of the mean. Indicates significant different (p ≤ 0.05) using the Student’s *t* test. Different upper-case letters indicate significant differences between cell populations within the different indication time within the same stress treatment.
Figure 5

Changes in viable cell counts (differences between counts at 24, 48, 72 h, in log CFU ml of Lb. *acidophilus* at different NaCl concentration. Bars indicate standard deviation of the mean. Indicates significant different (*p* ≤ 0.05) using the Student’s *t* test. Different upper-case letters indicate significant differences between cell populations within the different indication time within the same stress treatment.
Figure 6

Evolution of the cell populations during co-culture fermentation in PM

Different upper-case letters indicate significant differences; *different lower-case letters indicate do not significant* differences
Acidification rate during co-fermentation A formation of a dense clot was formed by 12 h and remained stable at the end of co-fermentation process.