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Isolation, identification, and characterization of lactic acidic bacteria isolated from the raw milk of a single-humped camel

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Abstract

The genus *Lactobacillus* contains several Gram-positive bacteria that are microaerophilic or facultatively anaerobic. These are the main groups of lactic acid bacteria. Some lactobacilli are used in the production of yogurt, cheese, and other fermented products. The purpose of this study was to isolate, identify, and characterize the technological characteristics of lactic acid isolated from a single-humped camel in the city of Tabas, Iran,by standard technological properties tests. From a total of 12 raw milk samples, 60 isolates were investigated by morphological and Gram studies. Thirty-six isolates of catalase-negative and Gram-positive selected for genus identification were compared using the Bergey Book. All examined isolates were analyzed to determine the carbohydrate fermentation pattern and CO_2 production. The results showed that 23 isolates of *Lactobacillus* (with a 63.88% frequency) were found in five groups, including *Lactobacillus gasseri* isolates (L1-L7), *Lactobacillus gallotix* isolates (L8-L13), *Lactobacillus xiangfangensis* isolates (L14-L16), *Lactobacillus salivarius* isolates (L17-L21), and *Lactobacillus paracasei* isolates (L22, L23). Additionally, five *Pediococcus* isolates, P1-P5 (13.99%), and eight *Enterococcus* isolates, E1-E8 (2.22%), were obtained. Seven isolates were evaluated for molecular identification using 16S rRNA. Finally, five *Lactobacillus*, one *Pediococcus*, and one *Enterococcus* were identified. These seven isolates were examined by complementary tests including proteolytic, lipolytic and autolytic activities, and antibiotic and acid resistance. Two isolates, K030508 (*L. salivarius*) and K020704 (*L. xiangfangensis*), exhibited the highest potential for future commercial use as a starter culture.

1. Introduction

Lactic acid bacteria (LAB) are a diverse group of non-motile, Gram-positive, microaerophilic bacteria that are genetically and ecologically diverse. Lactic acid bacteria include genera belonging to the Firmicutes phylum such as *Enterococcus*, *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Oenococcus*, *Lactococcus*, *Streptococcus*, *Weissella*, etc. within the order *Lactobacillales* and the anaerobic *Bifidobacterium* genus belonging to the Actinobacteria phylum (Hatti-Kaul et al. 2018). Some starter bacteria, such as *Lactobacillus*, *Lactococcus, and Pediococcus*, which belong to the lactic acid bacteria, are naturally present in milk and are

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capable of converting lactose to lactic acid, which produces a savory flavor as well as clotting tissue in the product. In general, starter microorganisms are added to dairy products to create fundamental changes in the product`s aroma, taste, and texture. By converting lactose to lactic acid, the pH of the milk would be reduced to 4.6. This would not only prevent the growth and survival of pathogenic bacteria but also break down the milk protein, casein. This process occurs due to the collapse of protein particles after reaching the isoelectric point. Aromatic diacetyl is the final substance obtained during this procedure (Rahmati 2019).

Various biotechnological companies are currently engaged in the production of industrial starter cultures to be sold to the dairy industry to produce various fermentation products. Factories produce thousands of dairy products daily to meet the growing population's increased consumption of dairy products. This has necessitated the use of more LAB to meet the demand. Statistical surveys have shown that the dairy industry in Iran is largely dependent on the supply of these starter cultures from abroad, and according to the latest annual information, three million dollars is dedicated to importing starter strains from other countries (Iran Customs, 2014). This dependency will bring limitations if restrictions on the sale of starter to Iran are imposed. Moreover, with the general promotion of sterilized food being healthier, the consumption of traditional dairy products has declined, suggesting that the native starter strains of Iran are being destroyed over time, which can be considered as irreparable national harm.

The purpose of this study was the isolation and molecular identification of lactic acid starter isolates from raw milk of single-humped camels in Tabas, Iran, by standard technological properties tests, including lipolytic and proteolytic activities, as well as determining the antibiotic resistance pattern. In this way, isolates with the highest potential would be identified for use as starter cultures on a commercial scale in Iran's dairy industries in the near future.

2. Materials and methods

2.1 Sampling

In this study, 12 raw milk samples (50 ml) from a single-humped camel were collected from camels in the city of Tabas (South Khorasan Province, east of Iran) in August 2018. All samples were stored at 4 °C temperature and transferred to the laboratory for isolation and identification as well as supplementary tests. All sampling and transfer steps were carried out in sterile conditions.

2.2 Isolation, enrichment, and biochemical tests

First, the milk samples were thoroughly shaken to homogenize the milk. Then, 5 mL of each sample was added to 45 ml of MRS (de Man, Rogosa, Sharpe) broth medium to enrich the LAB (Simpsone et al. 2006). Samples were incubated at 37 ° C for 72 h, and a candle Jar was used to provide microaerophilic conditions. In order to prepare a 10-1-10-7 concentration, the serial dilution method was used in peptone water (Merck, Germany) on a cultured pour plate of MRS agar incubated at 37 ° C for 48 h. Finally, a number of typical colonies isolated randomly were investigated for morphological characteristics, such as appearance, size, and convexity. The colonies were purified three times on MRS agar for further assurance.

2.3 Biochemical and phenotypic identification of isolates

For this purpose, the colonies that were differentiated in the colony appearance were used to determine the type of Gram and other biochemical properties, such as catalase and oxidase activity. In general, samples that were Gram-positive and catalase and oxidase negative were identified as LAB. Grown isolates were detected at the genus level for the CO2 production from glucose fermentation (MRS broth without meat extract and containing 50g/l glucose), salt tolerance (6.5 and 18% NaCl), growth at 10, 15, 45 °C, and growth at pH 6.9 and 4.4 (Alhaag et al. 2019; Rahmati. 2017; Sneath et al. 1986; Harrigan & McCance 1976).

Carbohydrate fermentation patterns were used to identify the lactobacillus isolates. Nine different carbohydrates, including galactose, lactose,

trehalose, mannitol, raffinose, maltose, arabinose and ribose, were employed. 50 µL of bacterial suspension was added to a MRS Broth medium base without glucose or beef extract and containing phenol red (0.5g/l) as the indicator (Rahmati 2017). Lastly, to maintain anaerobic conditions, 1 ml of liquid paraffin (Rospolimer Co, Iran) was added to the surface of the tubes. All samples were incubated at 10, 15, and 45 °C for 72 h. Any changes in the color of the phenol red to yellow in the mediums shows the isolates ability for carbohydrate fermentation and acid production. The production of CO2 from the fermentation of various carbohydrates indicates whether the isolates are homofermentative or heterofermentative. A total of 60 isolates were selected for biochemical and Gram tests, 36 isolates were identified to genus by their ability to grow in different temperature, pH, and salt concentrations. Finally, seven confirmed isolates were studied in the next phase by supplementary technological tests.

2.4 Identification of the isolates with16S rRNA

The Araujo method (Simpsone et al. 2006), as a lysosomal digestion pathway, was used to isolate and purify the DNA of the isolates. The extracted DNA was assessed by qualitative and quantitative studies. For this purpose, the NanoDrop machine (THERMO ND 2000, USA) was used for quantitative control. In the next step, agarose gel 1% (Major Science, Taiwan) was employed for DNA qualitative control, and then all samples were prepared for PCR. The two primers used in this study included (616V, 5`-AGAGTTTGATYMTGGCTCAG-3`) and (630R 5`-CAKAAAGGAGGTGATCC-3`) (Ehrmann et al. 2003). The PCR was carried out with 0.4 µm of the primers, 50 ng/µl of the DNA template, and 12.5 µl of the master mix (Cinnagen Co, Iran). A final volume of 25 µl was reached with sterile distilled water. Gene amplification was initiated by primary denaturation at 94 °C for 180 seconds in a single cycle, followed by 30 cycles at 94 °C for 15 seconds, 53 °C (annealing temperature) for 40 seconds, and 72 °C for 40 seconds. The quality of the PCR products was evaluated by electrophoresis on 1.5% TBE agarose gels with a ladder of 100 bp.

2.5 Sequencing the multiplied fragments

A specified volume of purified product placed in a microtube was sent to the Macrogen company (Macrogen, South Korea). Using this method, all isolates were sequenced using an ABI 3730XL DNA Sequencer and the Sanger method. Practical software Chromas Lite 2.1.1. (Technelysium Pty Ltd, South Brisbane, AU) and BioEdit 7.2.5 helped to ensure the integrity of the sequences sent from the company. The available BLAST (Basic Local Alignment Search Tools) bioinformatics software at the NCBI website was used to align the isolates and determine the sequence similarity of the 16S rRNA fragments.

2.6 Assessment of the technological characteristics

2.6.1 Proteolytic and lipolytic activity

The proteolytic activity of the isolates was investigated by casein hydrolysis on MRS agar containing 10% Skim Milk powder. 1% of the bacterial suspensions at a concentration of 0.5 newly cultured McFarland was added to the base culture medium (Swearingen et al. 2001). Then, all plates were incubated in facultative anaerobic conditions at 37 ° C for 24 h. Altogether, isolates containing protease enzyme that grew in the medium were distinguished from the other isolates that lack this activity by creating a clear and transparent zone in the medium.

For lipase activity, 1 ml of enzymatic solution was added to 3ml of phosphate buffer 0.2M with pH7 and calcium chloride 0.1 M. Then, 5ml of the substrate (olive oil and polyvinyl alcohol at a ratio of 3:1) was added and shaken for 15 minutes. The reaction was stopped by adding 20 ml of acetone-ethanol. Finally, three drops of 1% phenolphthalein were added to the solution and titrated with 0.05 normal NaOH. For the control solution, all the above steps were performed except adding the enzymatic solution. A unit of lipase activity (LU) is the amount of enzymatic

activity that releases one micromole of fatty acid per minute at 37 ° C and phosphate buffer at pH7 from the substrate (Davati & Zibaee 2017).

2.6.2 Bile resistance

In this test, MRS broth containing 0.3% bile oxalate and 100 µL of the bacterial suspension was added to the tubes. To determine the optical density (OD) of the isolates, all samples were inserted in a spectrophotometry apparatus (Jenus, UV-1200), and ODs were read at 650 nm (Piraino et al. 2008). In the next step, the samples were incubated under facultative anaerobic conditions for 8 h at 37 ° C; the samples' ODs were investigated once more to determine the rate of bile resistance. The resistance of isolates to bile oxalate was interpreted according to the standard INSO 19459 for probiotic bacteria, which states that the inhibitory rate of (Cinh) should not be more than 0.4 (Institute of Standards and Industrial Research of Iran, 2012).

2.6.3 Antibiogram test

The antibiogram test of the isolates was performed by the disc diffusion (Kirby-Bauer) method, and the results of the test were compared and interpreted as Sensitive, Semi Sensitive, or Resistant (Table1). The eight antibiotics used in this test (Padtan Teb Co, Iran) included erythromycin (15 µg), gentamicin (10 µg), kanamycin (10 µg), chloramphenicol (30 µg), ampicillin (10 µg) vancomycin, (30 µg), clindamycin (10 µg), and tetracycline (30 µg). In the next step, 100 µl of the bacterial suspension was added to each medium. Antibiotic disks were placed on an MRS agar medium, and all isolates were incubated anaerobically at 37 °C for 24 h. If the diameter of the inhibition zone (no bacterial growth) around the disc is greater than the specified amount determined for each antibiotic, the isolate is considered sensitive to the antibiotic (Rahmati 2018a).

2.6.4 Tolerance of the bacteria against acidic condition

To accomplish this test, MRS broth was employed, and the medium pH was separately adjusted to 2.5-0.4. Generally, 100 µl of bacterial suspension was added to the medium, and samples were incubated at 37 °C for 4 h. Afterward, isolates were streaked on MRS agar and incubated at 37 °C for 72 h in microaerophilic conditions. At the end of the test, the ability of isolates to grow in an acidic environment was interpreted (Chateau et al. 1994).

3. Results and Discussion

3.1 Isolation, screening and identification of isolates to genus

In this study, twelve samples of single-humped camel raw milk were randomly selected in Tabas city. Sixty isolates were selected according to the results of Gram staining and biochemical tests. The observations showed that most isolates were obtained from dilutions 10-4 and 10-10. Also, the isolate that grew at 44 °C and 25 °C was much less than the isolate at 37 °C.

Table 1: Determining the sensitivity to antibiotics (mm).

Degree of sensitivity	**Tetracycline**	**Gentamicin**	**Chloramphenicol**	**Kanamycin**	**Clindamycin**	**Ampicillin**	**Vancomycin**	**Erythromycin**
Resistant	X<14	X<12	X<12	X<13	X<14	X<11	X<9	X<13
Semi Sensitive	15-18	13-14	13-17	14-17	15-20	12-13	10-11	14-17
Sensitive	X>19	X>18	X>18	X>18	X>21	X>14	X>12	X>18

All 60 isolates were evaluated on morphological characteristics observed under a microscope. Generally, the lactobacillus genus was considered to be gram-positive bacilli with single, pair, or chain forms, non-spore, and motility with catalase and oxidase-negative. In addition, other gram-positive cocci isolate, mostly in pair forms, non-spore, and motility, as well as catalase and oxidase negative known as Pediococcus with white to gray colonies. *Enterococcus* was also considered gram-positive cocci, non-spore with catalase negative and positive oxidase in pair and chain forms. Thirty-six catalase negative and gram-positive isolates were evaluated to identify the genus (Sneath et al. 1986). The highest number of isolates with 63.88 % abundance (23 isolates) belongs to *Lactobacillus*, 13.99 % abundance (5 isolates) belongs to *Pediococcus*, and 22.22 % abundance (8 isolates) belongs to *Enterococcus*. The results are shown in Table 2 for further details.

Table 2. Number and abundance of isolates.

	Enterococcus	***Lactobacillus***	***Pediococcus***
Isolates	E1-E6	L1-L23	P1-P5
Number	8	23	5
Abundance (%)	22.22	63.88	13.99

The results obtained from the fermentation pattern of various carbohydrates and the ability to produce CO2 from glucose were interpreted with Bergey's manual guide (1984-1989, 2009), and the isolates were identified to the species level. After analyzing the results, 23 isolates of *Lactobacillus* in 5 different groups, including *L. gasseri* (L1-L7), *L. gallotix* (L8-L13), L. xiangfangensis (L14-L16), *L. salivarius* (L17-L21), and *L. paracasei* (L22, L23). The results of isolates identified to the species are summarized in Table 3.

Table 3. Identification of isolates based on phenotypic characteristics and abundance of isolates.

Isolates	**Isolates code**	**Bacterial species**	**Number**	**Abundance**
L1-L7	K030701	*Lactobacillus gasseri*	7	19.44
L8-L13	K0103012	*Lactobacillus helveticus*	6	16.66
L14- L16	K020704	*Lactobacillus xiangfangensis*	3	8.33
L17-L21	K030508	*Lactobacillus salivarius*	5	13.88
L22,L23	K030301	*Lactobacillus paracasei*	2	5.55
P1-P5	K010205	*Pediococcus acidilactic*	5	13.88
E1-E8	K010206	*Enterococcus faecium*	8	22.22

In the last step, prior to the technological characteristics tests, the seven isolates were evaluated for molecular identification of the LAB isolates by 16S rRNA. The sequence of bacteria after searching in BLAST showed the maximum synchronization with the sequences corresponded to 97- 100%. Table 4 shows the result of the identification of the isolates.

Table 4. Results of molecular identification of isolates and their similarity to the BLAST.

Isolates code	**Identified isolates**	**Similarity rate**
K030508	*Lactobacillus salivarius*	100%
K0103012	*Lactobacillus helveticus*	99%
K020704	*Lactobacillus xiangfangensis*	100%
K030701	*Lactobacillus gasseri*	99%
K030301	*Lactobacillus paracasei*	99%

K010205	*Pediococcus acidilactic*	100%
K010206	*Enterococcus faecium*	99%

After sequencing, the phylogenetic analysis of isolated strains was compared to close strains sequences available in the GenBank database (http://www.ncbi.nlm.nih.gov) and through the EzTaxon database (http://www.extaxon.org) using the BLASTn program and then coordinating and calculating the evolutionary distances of the sequences. A phylogenetic tree was drawn using the neighbor-joining algorithm in CLustalX and MEGA 6 software. The accuracy of the tree branches was assessed using bootstrap analysis with 1000 times of resampling (Fig. 1) (Salari et al. 2020).

In a similar study conducted by Ghobadi Dana et al. in 2012, two strains, *Lactobacillus crustorum-125* and *Lactobacillus crustorum-90*, were identified using molecular techniques and a database from native dairy products of three different provinces in Iran to have the highest potential to be employed as dairy starter cultures. Aplevicz et al. (2014) also isolated and identified strains of *L. paracasei* and Saccharomyces cerevisiae from a Brazilian native product, sourdough, in a similar study.

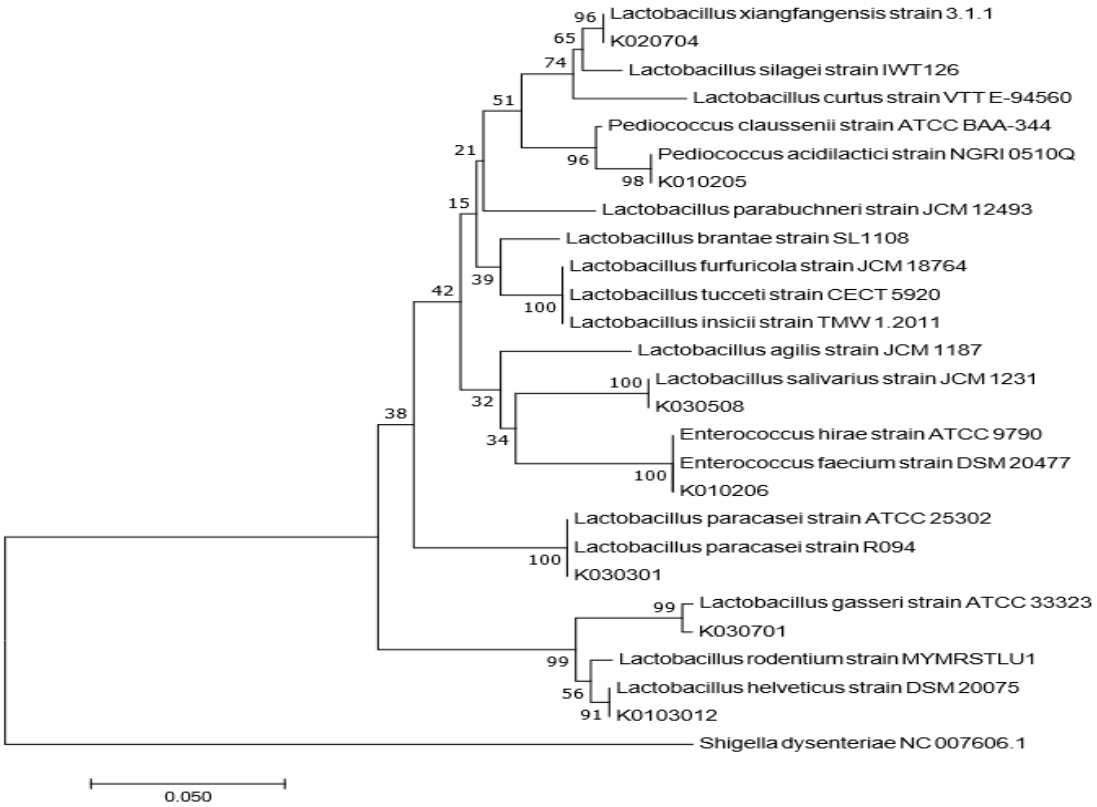

Figure 1. Phylogenetic tree of *Lactobacillus* and *Enterococcus* species.

3.2 Assessment of technological properties of isolates

3.2.1 Proteolytic and lipolytic abilities

Generally, the achievement of high-quality dairy products relies on the proteolytic ability of starter strains (Ayad et al. 2004). Due to the active role of proteolysis to improve the taste, the proteolytic properties of starter cultures are considered a key factor in phenotypes because the production of peptides and amino acids results from the activity of the protease enzyme. Free amino acids and complex peptides influence the flavor and aroma by secondary catabolic reactions. Vuillemard et al. (1986) argued that when the diameter of the proteolyzed zone ranges from 15 to 21 mm, it indicates that the isolate is capable of hydrolyzing the protein in the milk. Based on this standard, two strains, *L. salivarius* (K030508) and *L.*

xiangfangensis (K020704), showed proteolytic ability in the MRS agar medium. The rest of the isolates had poor activity or no enzymes for protein hydrolysis.

The most important lipolytic activity of the strains is related to the cheese industry, since the special texture and flavor of the cheese is, to a large extent, dependent on this starter culture activity (Stark & Scheib 1935). Table 5 summarizes the results of the lipolytic test, and only 4 of the seven isolates studied in this study had lipolytic properties, and the K020704 isolate (*L. xiangfangensis*) revealed the most lipolytic activity.

Table 5. The results of lipolytic activity of isolates.

Isolates	Lipolytic activity (unit/ml)
K030508	10
K030301	8
K030701	8.5
K020704	11.5

Bacteria have different enzymes that are used to produce various products in factories. Protease is one of the most important and key enzymes in the dairy industry. In fact, one of the most important factors in the production of a pleasant fragrance and taste in dairy products, such as cheese, yogurt, and sourdough, is the proteolytic ability of probiotic starters. In fact, this aroma is due to the presence of diacetyl and complicated peptides produced by the secondary metabolites in the biochemical cycles of bacteria in the stabilization phase. Only two isolates, *L. salivarius* and *L. xiangfangensis*, were able to produce the areola in the culture medium; the rest of the isolates had weak or non-enzyme activity for protein hydrolysis. This result was relatively in agreement with other studies. In two studies conducted by Rahmati (2018b, 2017)], some strains of *Lactococcus lactis*, *Lactococcus plantarum*, and *Lactococcus garvieae*, along with *Lactobacilli* strains such as *L. cassei* and *L. plantarum*, exhibited proteolytic properties with the formation of an inhibition zone with a diameter of more than 13 mm. Also, Ma et al. (2012) isolated several *Lactobacillus* strains with protease enzyme that had high potential for the production of the enzyme.

3.2.2 Evaluation of Bile salt resistance

The results of this study show that seven LAB isolates based on the above-mentioned criteria showed a good resistance. Meanwhile, *P. acidilactic* (K010205) and *L. helveticus* (K0103012) isolates exhibited the least and the most activity, respectively (Table 6).

Table 6. Resistance to bile salt of seven isolates of *L. helveticus* (K0103012), *P. acidilactic* (K010205), *L. paracasei* (K030301), *L. xiangfangensis* (K020704), *L. gasseri* (K030701), *E. faecium* (K010506), and *L. salivarius* (K030508).

Isolates	C*inh*
K0103012	0.3870±0.001
K010205	0.0200±0.01
K030301	0.3520±0.006
K020704	0.3555±0.0035
K030701	0.3460±0.003
K010506	0.1605±0.0075
K030508	0.1165±0.0065

The human gallbladder (a small pouch that sits just under the liver) plays a non-specific inhibitory effect on the growth of bacteria in the intestine, which is actually a type of immune system in the body. The concentration of bile salts in the gastrointestinal tract is 0.3% (w/v) provide good conditions for the evaluation and selection of bile-resistant strains (Chateau et al. 1994). A comprehensive study by Chateau et al. was carried out on 38 isolates from the *Lactobacillaceae* family. Their results revealed that 19 isolates showed a one-hour delay in growth due to the gradual vicinity of bile salts with a concentration of 0.3% to isolates cultured in the blank free bile salt MRS medium. These results were obtained by the optical density of isolates at 650 nm wavelength by spectrophotometry, and the delay in the growth of isolates was absolutely obvious. Mainly, the growth delay, based on the Chateau model, is divided into four different types. The first type refers to those isolates that indicate a delay in growth below 15 minutes, known as

resistant isolates. The second group includes isolates with relatively high salt tolerance, which usually indicate growth retardation of fewer than 30 minutes. Those isolates between 45 and 60 minutes of growth delay include semi sensitive isolates. Sensitive isolates also have a delay in growth for more than an hour. According to the standard national organization, No. 19459, if the tolerance of the acidity changes in the resistance test to bile salts for probiotic strains is less than or equal to 0.4, then that isolate is considered to be resistant (Fguiri et al. 2016). Generally, in this research, all seven isolates from a single-humped camel revealed resistance toward bile salt and acidity shifts.

3.2.3 Antibiotic resistance of isolates

The LAB isolates can potentially have a resistance gene to various antibiotics and also transfer the resistance gene to other isolates. Antibiotic resistance of starter cultures in dairy products may cause antibiotic resistance in humans (Fguiri et al. 2016). Therefore, the bacteria used in the dairy and fermentation industry should be free from any antibiotic resistance gene. In this research, the resistance of isolates was investigated through the disc diffusion method and the assessment of growth ability in the proximity of antibiotics (the presence or absence of an inhibition zone around the disk). According to the results found in Table 7, all isolates were sensitive to ampicillin, and the isolate *L. gasseri* (K030701) was resistant to vancomycin and gentamicin. Also, *L. paracasei* isolate (K030301) was reported resistant to kanamycin, and the other isolates were sensitive and semi-sensitive to four different antibiotics.

Table 7. Results of isolation resistance to eight different antibiotics. **Legend:** (S): Sensitive, (SS): Semi Sensitive, (R): Resistant. All measurements are based on millimeter (mm).

Isolates	Erythromycin	Vancomycin	Ampicillin	Clindamycin	Kanamycin	Chloramphenicol	Gentamicin	Tetracycline
(L8)K0103012	17 (SS)	14 (S)	24 (S)	30 (S)	16 (SS)	30 (S)	15 (S)	20 (S)
(P2)K010205	16 (SS)	11 (SS)	32 (S)	22 (S)	17 (SS)	20 (S)	14 (SS)	17 (SS)
(L22)K030301	21 (S)	10 (SS)	18 (S)	21 (S)	12 (R)	28 (S)	13 (SS)	20 (S)
(L15)K020704	15 (SS)	13 (S)	15 (S)	24 (S)	21 (S)	24 (S)	14 (SS)	15 (SS)
(L3)K030701	16 (SS)	9 (R)	16 (S)	22 (S)	15 (S)	21 (SS)	12 (R)	19 (S)
(E7)K010206	20 (S)	9 (S)	17 (S)	25 (SS)	19 (S)	18 (S)	15 (S)	17 (SS)
(L18)K030508	19 (S)	12 (S)	18 (S)	24 (S)	16 (SS)	22 (S)	16 (S)	18 (SS)

If the starter strains in the dairy industry contained an antibiotic resistance gene, then it is possible for these genes to be transmitted to other bacteria in the intestine when these bacteria enter the lumen tract in the human digestive system through dairy products such as yogurt and cheese, which creates ever-increasing antibiotic resistance to bacteria in the human body. Therefore, it should be noted that a suitable strain for application in the fermentation industry should not have any resistance gene against antibiotics (Amor et al. 2007; Fguiri et al. 2016). Strains that carry a gene or antibiotic resistance vector are not allowed to be used in the food and fermentation industry. In this study, antibiotic resistance patterns of the isolates were evaluated against eight different antibiotics. In the

end, a huge number of isolates were sensitive and semi-sensitive to antibiotics, and only isolates L3 and L22 were resistant to vancomycin, gentamicin, and kanamycin, respectively. These results are in agreement with the results of Jini et al. (2011), which emphasized that all native strains were sensitive to most antibiotics, and only two strains revealed a relative resistance against cotrimoxazole and vancomycin.

3.2.4 Isolates resistance to acid and ability to live in the medium.

When milk or dairy product turn acidic due to the production of acid, the strains must exhibit an appropriate resistance to it. In this study, acid resistance of seven LAB isolates from single-humped camel milk were investigated at two pH 4 and 2.5. According to Iranian national standard No. 19459, associated with probiotic bacteria, when probiotics are placed at pH 4 and 2.5, the number of bacteria should not be less than 106 (Rahmati 2018). Using Prism software (GraphPad Prism 6), the analysis results and graph were plotted. Fig. 2 shows that the majority of isolates demonstrated an acceptable resistance to acid, and the highest and lowest acid resistance belongs to *L. salivarius* (K030508) and *L. paracasei* (K030301), respectively.

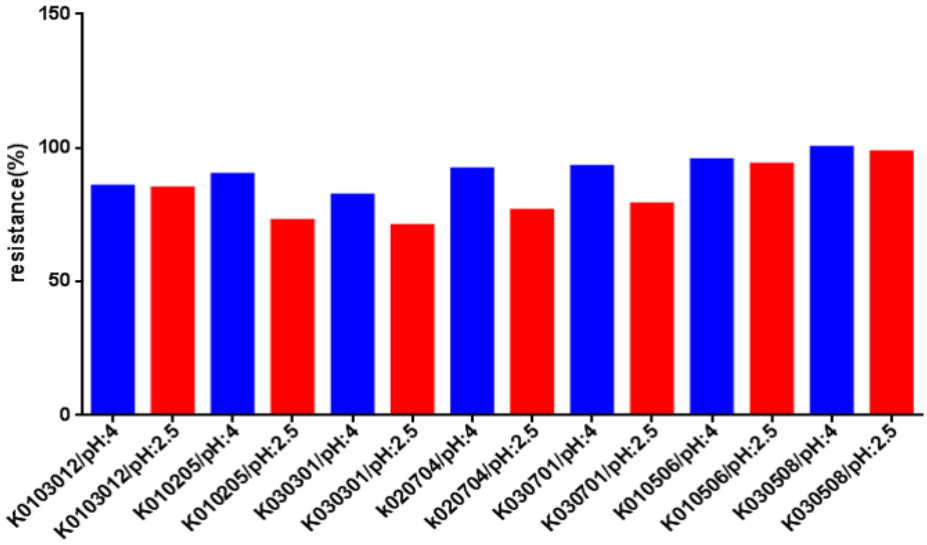

Figure 2. The results of resistance to acid at pH 4.0 and 2.5 of seven isolates of *L. helveticus* (K0103012), *P. acidilactic* (K010205), *L. paracasei* (K030301), *L. xiangfangensis* (K020704), *L. gasseri* (K030701), *E. faecium* (K010206), and *L. salivarius* (K030508).

A wide-ranging investigation was conducted on the determination of acid lactic bacteria resistance against acidity in recent years. More than thirty years ago, Conway et al. (1987) reported resistance to isolates against acidity. Similarly, Fernandez et al. (2003) in Spain and Ghobadi Dana et al. (2011) in Iran confirmed the existence of such resistant strains; in the second, a resistance of 60 to 80% of isolates from milk and yogurt was reported.

4. Conclusions

In this study, only two of the seven isolates, K030508 (*L. salivarius*) and K020704 (*L. xiangfangensis*), showed practical potential for use as starter cultures. Both isolates exhibited the least resistance to antibiotics, which is one of the main characteristics of a dairy starter. Only these two isolates showed a proteolytic ability in the culture medium, and they had the highest lipolytic activity of 11.5 unit/mL and 10.3 unit/mL, respectively. These two tests confirmed the ability of the isolates to produce aromatic and pleasant taste products. These two isolates have the potential to

be applied commercially in dairy factories, although they must be registered at the gene bank of microorganisms and be supported by further research and development teams.

Conflict of interest

Authors declare that there is no conflict of interest.

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Ethical approval

This article does not contain any studies with human participants or animals.

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