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Isolation of lactic acid bacteria with probiotic potential from bovine colostrum in livestock farms of Ramian Township in located in the north of Iran

Zahra Naemi¹, Hadi Koohsari^{*2}, Hamid Reza Pordeli³

1. Graduated student, Department of Microbiology, Gorgan branch, Islamic Azad University, Gorgan, Iran

2. Assistant Professor, Department of Microbiology, Azadshahr branch, Islamic Azad University, Azadshahr, Iran

3. Assistant Professor, Department of Microbiology, Gorgan branch, Islamic Azad University, Gorgan, Iran

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ABSTRACT

Antibacterial activity against gastrointestinal bacteria, tolerance to acidic conditions and bile salts are the main indicators of probiotics. The aim of this study was isolation of lactic acid bacteria with probiotic potential of bovine colostrum. Colostrum samples were cultured on selective culture. Identification of lactic acid bacteria carried out by biochemical tests of fermentation of sugars, growth at 10 and 45°C and 6.5% salt. Antagonistic effect of culture supernatant of isolates against native isolates *S. aureus*, *E. coli*, *P. aeruginosa*, *B. cereus* and standard strains *E. coli*, *P. aeruginosa*, *S. dysenteriae*, *S. aureus*, *B. cereus* and *E. faecalis* carried out by well method and tolerance of isolates to acidic conditions and bile salts were evaluated. *L. plantarum* was the most species of isolated lactic acid bacteria (36.32%). Culture supernatant of *L. plantarum* more than other isolates was showed antibacterial activity. None of the isolates were able to grow at pH 2, but *L. plantarum*, *L. casei*, *L. acidophilus*, *S. thermophilus* and *E. faecalis* were able to tolerate pH of 3. Also, *L. plantarum*, *L. casei*, *E. faecalis* and *E. faecium* were able to grow in presence of 0.3 and 1% bile salt. Colostrum as a traditional dairy food can be considered as a very suitable reservoir for isolation of lactic acid bacteria with probiotic potential. Due to significant antibacterial activity of *L. plantarum* and the ability to grow in acidic condition and various concentrations of bile salts, probiotic potential of this isolate was evaluated as desirable.

1. Introduction

Researchers have focused on the use of natural metabolites produced by bacteria for preventing the growth of pathogens. Probiotics are live microbes that are eaten with food and provide beneficial effects, such as improving the immune system, preventing the establishment and growth of pathogenic bacteria, reducing cholesterol absorption and reducing the risk of colon cancer. Probiotic bacteria have the ability

to replace in the digestive system and tolerating acid conditions of stomach and bile salts in the intestine (Frank et al., 2007).

The most important features of a probiotic microorganism include antagonistic effects on ten bacteria and the ability to living and activating in the digestive system, meaning the tolerating of the acidic conditions of the stomach and bile salts of intestine (Sanders, 2000; Pundir

*Corresponding author: Dr. Hadi Koohsari
Tel: (+98)17-35725925
E-mail hadikoohsari@yahoo.com

et al., 2013; Tambekar and Bhutada., 2010; Hiromi et al., 2004).

Lactic acid bacteria are the most common types of bacteria that have been introduced as probiotics.

Lactic acid bacteria are bacilli or cocci shaped, gram positive, non-spore, non-motile and catalase-negative bacteria whose main source of carbohydrate fermentation is lactic acid. Important lactic acid bacteria in the dairy industry belong to the genus *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus* and *Enterococcus*. Antibacterial activity of lactic acid bacteria can be due to acid production and pH reduction, the production of high molecular weight compounds such as bacteriosins and low molecular weight compounds such as hydrogen peroxide, carbon dioxide, and di-acetyl (Rattanchikunopon and Phuinkhachorn, 2010; Haghshenas et al., 2015; Serrano-Nino et al., 2016).

Traditional dairy products are complex ecosystems of a variety of lactic acid bacteria that are considered as a reservoir for the isolation of lactic acid bacteria with probiotic potential. Colostrum is the first milk that secretes after postpartum. This milk is made by the mammalian tubers at the end of pregnancy and is secreted at 24 to 96 hours of the postpartum. Colostrum has antibodies that protects the baby against diseases and has a lower lipid and higher protein than the normal milk. This food is a source of essential protein, fat, lactose, vitamin and mineral compounds and plays a vital role in the health of the baby. Compounds such as antibodies, lysozyme, lactoferrin, lactoperoxidase, and cytokines in the colostrum have antimicrobial properties (Ayar et al., 2016, Solomons et al., 2002; De Dea et al., 2011). Also, colostrum contains microbes, including lactobacilli and bifidobacteria, which may have antibacterial effects against pathogenic bacteria which can be used as an important reservoir for the isolation of bacteria with probiotic potential (Dubos et al., 2011; Khotimah et al., 2015; Ayar et al., 2016).

The colostrum is called in the local language of Shimak, Paleh, Faleh, Qrimakh and colostrum in the scientific language. Colostrum as a dairy food has been studied less in terms of a reservoir for isolation lactic acid bacteria with probiotic potential. The aim of this research was to isolate lactic acid bacteria from bovine colostrum with

the ability to inhibit ten gram-positive and gram-negative bacteria and ability to tolerate acidic conditions and bile salts.

2. Materials and Methods

2.1. Isolation of Lactic acid bacteria from colostrum samples

Colostrum samples from 4 traditional livestock farms in the Ramian township in Golestan province located in the north of Iran were collected in December 2018 and until the tests were kept in refrigerator of the microbiological laboratory of Azadshahr Islamic Azad University.

In order to enrichment of lactic acid bacteria, 1ml of colostrum sample was added to culture media of M17 broth (Himedia, India) and MRS broth (Conda Pronadisa, Spain) contains nystatin (50µ/ml) (Emad Darman Pars Co. Iran) (In order to prevention of yeasts growth).

After 24 hours of incubation at 37°C, samples were cultured on MRS agar (Conda Pronadisa, Spain), M17 agar (Himedia, India) and KAA (Liofilchem, Italia) in order to isolation of lactobacilli, streptococci and enterococci respectively. Plates were incubated in a candle jar for 48-72 hours at 37°C.

After, on suspicious colonies were performed gram stain and catalase test and colonies with morphology cocci and bacilli gram positive and negative catalase were purified on the MRS agar and subsequent experiments were performed on these bacteria.

For preservation of these isolates, high concentration of culture of each of them was prepared in a MRS agar containing 20% glycerol and stored in a freezer at -20°C (Greco et al., 2005; Torres Lianez et al., 2006).

2.2. Identification of isolated lactic acid bacteria

In order to identification of the isolates was used from biochemical tests including sugar fermentation of galactose (Merck, Germany), maltose (Conda Pronadisa, Spain), fructose (Merck, Germany), sucrose (Merck, Germany), raffinose (Merck, Germany), sorbitol (Merck, Germany), lactose (Merck, Germany), mannose (Merck, Germany), ramnose (Merck, Germany), and mannitol (Merck, Germany), along with the ability to grow at 10 and 45°C and in the concentration of 6.5% (Bhardwaj et al., 2012;

Davarpanah and Koohsari, 2017). The isolates bacteria to species level were approved according to the latest edition of the Bergey's Manual of Systematic Bacteriology (Finegold et al., 2009).

2.3. Preparation of culture supernatant of isolated lactic acid bacteria

Several colonies of lactic acid bacterial isolates were inoculated into MRS broth medium and covered with sterile liquid paraffin and incubated at 37°C for 4 days. Then paraffin was removed and the tubes were centrifuged at 4000g for 10min and to ensure the absence of bacteria the supernatant were passed through 0.22 µm sterile syringe filter (Jet-Biofil); and were kept inside of the sterile tube in the refrigerator until antibacterial testing (Aslam and Qazi, 2010; Pundir et al., 2013; Davarpanah and Koohsari, 2017).

2.4. Preparation of turbidity equal to 0.5 McFarland of tested bacteria

In this study, antibacterial effect of culture supernatant of lactic acid bacteria isolated from colostrum samples against 10 bacteria including of 4 native isolates and 6 standard strains evaluated. Native isolates include *Staphylococcus aureus* isolated from the nasal cavity of the carrier, *Escherichia coli* isolated from the stool, *Pseudomonas aeruginosa* isolated from water and *Bacillus cereus* were isolated from the soil. The standard strains used in this study included three types of gram-negative bacteria *Escherichia coli* (PTCC 1338), *Pseudomonas aeruginosa* (PTCC 1811) and *Shigella dysenteriae* (PTCC 1188) and three types of gram-positive bacteria are included *Staphylococcus aureus* (PTCC 1112), *Bacillus cereus* (PTCC 1154) and *Enterococcus faecalis* (PTCC 1778) which were provided from the Iranian Research Organization for Science and Technology (IROST) in a lyophilized form. Then, they recovered in BHI medium (Conda Pronadisa, Spain) for 24 h at 37°C in the microbiology laboratory of the Azadshahr branch, Islamic Azad University. The 24-hour culture of each bacterium were inoculated into Nutrient Broth (Merck, Germany) and it was incubated at 37°C for to obtain turbidity equal to

0.5 McFarland = 1.5×10^8 CFU/ml (Pundir et al., 2013; Cockerill et al., 2012).

2.5. Evaluation of antibacterial activity by well method

Antibacterial activity of culture supernatant of lactic acid bacteria isolated from colostrum samples was determined based on agar diffusion and by well method. In this method from microbial suspensions equal of 0.5 McFarland (equal to 1.5×10^8 CFU/ml) each of the 10 bacteria using sterile swabs on Muller Hinton Agar (Conda Pronadisa, Spain) uniform culture was prepared. Then, using by sterilized Pasteur Pipette wells at a diameter of 6 mm on the medium were created and 100 µl each of culture supernatant of lactic acid bacteria isolated were poured into these wells. The plates were then incubated at 37°C for 24-48 hours. After this time, using a millimeter ruler the diameter of inhibition zone was measured and recorded (Aslam and Qazi, 2010; Pundir et al., 2013; Davarpanah and Koohsari, 2017).

2.6. The ability to tolerate the acidic conditions of isolated lactic acid bacteria

In order to select acid resistant isolates, MRS broth was prepared with pH 1, 2, 3 and 4 using HCl 1N. Then the isolated lactic acid bacteria were inoculated into these MRS broth tubes and incubated at 37°C for 2-3 days. After this time from each tube was cultured on MRS agar and incubated at 37°C. The formation and appearance of colonies after 48 hrs indicates tolerate acidic conditions (Tambekar and Bhutada, 2010; Pundir et al., 2013).

2.7. The ability to tolerate the bile salts of isolated lactic acid bacteria

In order to evaluate resistance to bile salts in isolated lactic acid bacteria was used from Oxgall (Merck, Germany), a mixture of cholic acid (bile salts insoluble in water) and taurocholic acid (bile salts soluble in water). Since the concentration of bile salts at the beginning of the intestine 1% and in the intestine up to 0.3%, these two concentrations were used to investigate the resistance of isolates to bile salts (Hiromi et al., 2004).

In order to select bile salts resistant isolates, MRS broth with 1% and 0.3% oxgall was

prepared and each isolate was inoculated into these tubes and incubated for 48 hours at 37°C. After this time from each tube was cultured on MRS agar medium and incubated at 37°C. The formation and appearance of colonies after 48hrs indicates tolerance to bile salts (Tambekar and Bhutada, 2010; Pundir et al., 2013).

2. 8. Statistical Analysis

Data analysis was done by SAS software and charts and tables were performed with Excel 2010 software. The experiments were conducted in the form of a factorial design. All experiments were performed in three replicates and the mean values were reported. To investigate the main and interactions of colostrum samples and probiotic potential of lactic acid bacteria on antibacterial activity, statistical analysis of variance (ANOVA) of the experimental data was performed. Duncan's multiple experiment was conducted to examine the significant differences between the experimental variables on the mean values with a 99% confidence level.

3. RESULTS

3.1. Isolation and identification of lactic acid bacteria

In this study, lactic acid bacteria were isolated and purified from bovine colostrum and the isolates were identified using morphological and biochemical tests. According to the results of the experiments, 22 isolates from colostrum samples were isolated as shown in Table 1. The results of this study showed that among 22 isolates of lactic acid bacteria, the highest frequency was related to *Lactobacillus* genus with 54.54%. *Lactobacillus plantarum* (36.32%) and *Streptococcus thermophilus* (22.7%) were the most species of isolated lactic acid bacteria. The lactic acid bacteria isolated from colostrum samples are listed in Table 1.

3. 2. Antibacterial activity of lactic acid bacteria isolated from bovine colostrum samples against gram-positive and gram-negative bacteria

The mean diameter of the inhibition zone of each bacteria in the presence of culture supernatant of the lactic acid bacteria isolated in Table 3 is presented. As can be seen, lactic acid bacteria isolated from colostrum samples could

affect on the tested bacteria. In between, *L. plantarum* was able to inhibit the growth of all ten gram-positive and gram-negative bacteria. So that with the mean diameter of the inhibition zone of 13.5, 14, 15, 14, 18, 16.5, 13, 13.5, 15.5 and 13.5 mm, showed significant antibacterial activity against native isolates and standard strains of *S. aureus*, *B. cereus*, *P. aeruginosa*, *E. coli*, *S. dysenteriae* and *E. faecalis* respectively (Table 3).

Culture supernatant of *L. casei*, *L. mesenteroides* and *S. thermophilus* also were able to demonstrate significant antibacterial activity against the bacteria tested (Table 3).

Statistical analysis showed that the antagonistic effects of *L. plantarum*, *L. casei*, *S. thermophilus*, and *L. mesenteroides* on tested bacteria were higher than other isolates and had a significant difference with other isolates ($P < 0.05$).

Based on statistical analysis, gram-negative bacteria were more susceptible than gram-positive bacteria, so that *S. dysenteriae* showed the highest susceptibility and *S. aureus* and *E. faecalis* showed the highest resistance ($P < 0.05$).

Also, there was no significant difference between standard strains and native isolates in terms of susceptibility to culture supernatant of lactic acid bacteria ($P = 0.59$).

3.3. Tolerance of acidic condition and bile salts in lactic acid bacteria isolated from colostrum samples

The results of tolerance of acidic condition and bile salts by lactic acid bacteria isolated are presented in Table 3.

As it is seen, all isolated lactic acid bacteria were able to grow at pH 4, but only *L. plantarum*, *L. casei*, *L. acidophilus*, *S. thermophilus* and *E. faecalis* were able to tolerate pH 3. In addition to, none of the isolates were able to grow at pH 2 (Table 4). *L. plantarum*, *L. casei*, *E. faecalis* and *E. faecium* were able to grow in the presence of 0.3 and 1% bile salt (Oxgall) (Table 4).

Table 1. Diagnostic table of isolated lactic acid bacteria based on biochemical test

Tests Isolate	Growth at 10 °C	Growth at 45 °C	Growth at 6.5% NaCl	Sugar Fermentation										number (Percent)	
				Galactose	Fructose	Raffinose	Lactose	Maltose	Sucrose	Sorbitol	Mannose	Rhamnose	Mannitol		
<i>L. plantarum</i>	-	-	-	+	+	+	+	+	+	+	+	+	+	+	8 (36.32)
<i>L. casei</i>	-	+	-	+	+	+	+	+	+	+	+	+	+	+	1 (4.54)
<i>L. acidophilus</i>	+	-	-	+	+	+	-	+	+	+	+	+	+	+	1 (4.54)
<i>L. helveticus</i>	+	-	-	-	-	-	+	+	+	+	-	-	-	-	1 (4.54)
<i>L. rhamnosus</i>	+	+	-	+	+	+	+	+	+	+	+	+	+	+	1 (4.54)
<i>Lactococcus lactis ssp cremoris</i>	+	-	-	-	-	-	+	-	-	-	-	-	-	-	1 (4.54)
<i>S. thermophilus</i>	-	+	-	-	-	+	+	+	+	+	+	+	+	+	5 (22.7)
<i>E. faecalis</i>	+	+	+	+	-	-	+	+	+	+	-	+	-	-	2 (9.09)
<i>E. faecium</i>	+	+	+	-	-	+	-	-	+	+	-	+	-	-	1 (4.54)
<i>L. mesenteroides</i>	+	+	-	+	-	-	+	+	+	+	+	-	-	-	1 (4.54)
Total														22 (100)	

Table 2. Types and number of lactic acid bacteria isolated from from colostrum samples

NO. Livestock	Number of lactic acid bacteria isolated	Lactic acid bacteria	
		Number	Type of Isolate
1	5	3	<i>L. plantarum</i>
		1	<i>S. thermophilus</i>
		1	<i>L. rhamnosus</i>
2	7	3	<i>L. plantarum</i>
		2	<i>S. thermophilus</i>
		1	<i>L. mesenteroides</i>
		1	<i>L. casei</i>
3	4	2	<i>S. thermophilus</i>
		1	<i>L. acidophilus</i>
		1	<i>Lactococcus lactis ssp cremoris</i>
4	6	2	<i>L. plantarum</i>
		2	<i>E. faecalis</i>
		1	<i>L. helveticus</i>
		1	<i>E. faecium</i>

Table 3. The diameter of the inhibition zone of gram positive and gram negative bacteria in the presence of the culture supernatant isolated lactic acid bacteria (mm)

Pathogen Bacteria LAB	<i>S. aureus</i> (N)	<i>S. aureus</i> (S)	<i>B. cereus</i> (N)	<i>B. cereus</i> (S)	<i>P. aeruginosa</i> (N)	<i>P. aeruginosa</i> (S)	<i>E. coli</i> (N)	<i>E. coli</i> (S)	<i>S. dysenteriae</i>	<i>E. faecalis</i>
<i>L. plantarum</i>	13.5±0	14±1	15±0	14±1	16.5±1	18±1	13±0	13.5±0	15.5±0	13.5±0
<i>L. casei</i>	-	-	-	12±1	13±1	-	9±0	9.5±0	14±1	9±0
<i>L. acidophilus</i>	-	9.5±0	12±1	-	-	-	-	9.5±0	11.5±0	9±0
<i>L. helveticus</i>	-	9±0	-	8±0	-	-	-	-	14±0	-
<i>L. rhamnosus</i>	-	-	-	9.5±0	-	9±1	-	-	12±1	-
<i>Lactococcus lactis</i> <i>ssp cremoris</i>	-	9±0	-	9±0	-	9±0	-	-	11.5±0	-
<i>S. thermophilus</i>	-	-	9±0	12±1	11±1	14±1	9±0	9±0	12±1	9±0
<i>E. faecalis</i>	-	-	-	-	-	10±0	-	-	11±1	-
<i>E. faecium</i>	-	10±0	-	-	-	10±0	-	-	12±1	-
<i>L. mesenteroides</i>	10±1	13.5±0	12±0	11±1	14±0	13±1	12±1	12±1	14±1	11±1

N: native isolate

S: standard strain

LAB: lactic acid bacteria

Table 4. The ability to tolerate acidic conditions and bile salts of isolated lactic acid bacteria from colostrum samples

Isolated LAB	Varying pH			Bile Salts Concentration (%)	
	2	3	4	0.3	1
<i>L. plantarum</i>	-	+	+	+	+
<i>L. casei</i>	-	+	+	+	+
<i>L. acidophilus</i>	-	+	+	-	-
<i>L. helveticus</i>	-	-	+	-	-
<i>L. rhamnosus</i>	-	-	+	-	-
<i>Lactococcus lactis</i> <i>ssp cremoris</i>	-	-	+	-	-
<i>S. thermophilus</i>	-	+	+	-	-
<i>E. faecalis</i>	-	+	+	+	+
<i>E. faecium</i>	-	-	+	+	+
<i>L. mesenteroides</i>	-	-	+	-	-

4. Discussion

The search for probiotic bacteria in traditional products provides the potential for introducing new strains for exploitation in the dairy industry. Antibacterial activity against gastrointestinal pathogen bacteria, tolerance to acidic conditions and bile salts tolerance is of the probiotic indicators.

4.1. Antagonistic activity of lactic acid bacteria isolated from bovine colostrum samples

As mentioned, culture supernatant of *L. plantarum* compared to isolates was showed to

be antibacterial activity against all tested bacteria, including standard strains and native isolates. Also *L. casei*, *S. thermophilus* and *L. mesenteroides* showed antibacterial activity against tested bacteria.

Significant antibacterial activity of *L. plantarum* isolated from dairy products has also been reported in similar studies (Haghshenas et al., 2015; Gupta and Srivastava, 2014; Ryu et al., 2014).

Forestier et al. (2001) showed antibacterial activity of *L. casei* subspecies of *rhamnosus* against a wide range of gastrointestinal pathogens (Forestier et al., 2001).

In the study of Serrano-Nino et al. (2016) isolated *L. mesenteroides* was able to prevent the growth of gastrointestinal pathogenic bacteria such as *E. coli*, *S. typhi* and *S. aureus* and due to the tolerance of digestive enzymes, acid conditions (pH = 2-3), attachment to intestine and growth at 40°C along with two isolates of *L. fermentum* and *L. delbrueckii* were introduced as probiotic bacteria (Serrano-Nino et al., 2016).

In order to isolation and identification of Lactobacilli with probiotic potential from traditional Iranian dairy products, Tajabadi Ebrahimi et al. reported the most antagonistic activity against intestinal pathogenic bacteria from *L. plantarum*, *L. brevis* and *L. casei* (Tajabadi Ebrahimi et al., 2011).

In the study of Salehi (2013) with the aim of investigating the antagonistic activity of lactobacilli isolated from traditional food products of western and eastern provinces of Iran against *E. coli* and *S. aureus*, among isolates *L. plantarum* and *L. casei* showed the most antibacterial activity (Salehi, 2013).

The antibacterial activity of lactic acid bacteria can be due to acid production and pH reduction as well as to the production of high molecular weight compounds such as bacteriosins and low molecular weight compounds such as hydrogen peroxide, carbon dioxide, and diacetyl (Rattanchikunopon and Phuinkhachorn, 2010; Haghshenas et al., 2015; Serrano-Nino et al., 2016).

Another results of the present study was the higher sensitivity of the gram-negative bacteria to culture supernatant of lactic acid bacteria isolated. This has been reported in similar studies (Tambekar and Bhutada, 2010; Serrano-Nino et al., 2016; Haghshenas et al., 2015; Obadina et al., 2006).

In study of Serrano-Nino et al. (2016) the gram-negative bacteria of *E. coli* showed a high sensitivity to four lactic acid bacteria isolated from milk and human colostrum, and *S. typhi* was resisted to culture supernatant only one of the isolates, while none one of the isolates was able to prevent the growth of gram-positive bacteria of *L. monocytogenes*, and only one of the isolates was able to inhibit of the gram-positive bacteria of *S. aureus* (Serrano-Nino et al., 2016).

Haghshenas et al. (2015) also have reported more sensitivity of gram-negative bacteria such as *E. coli* and *S. typhimurium* compared to gram-

positive bacteria of *B. cereus* and *L. monocytogenes* to the produced metabolites by 17 lactic acid bacteria isolated from bovine colostrum samples. The researchers found that this sensitivity in gram-negative bacteria was related to the thinner cell wall of gram-negative bacteria and the sensitivity of these bacteria to acidic compounds (Haghshenas et al., 2015).

Mack et al. (1999) stated in their report that the use of *L. plantarum* can reducing of gram-negative enterobacteriaceae in the intestines because it by producing bacteriosins prevents the binding of *E. coli* to the intestinal wall (Mack et al., 1999).

Miyamoto et al. (2000) identified bacteriocin production as the main cause of antibacterial activity of lactobacilli against gram-negative bacteria (Miyamoto et al., 2000).

In general, the more resistance of gram-positive bacteria to metabolites produced by lactic acid bacteria can be attributed to the cell wall of these microorganisms. Due to the thickness of the cell wall and the high number of peptidoglycan layers in the gram-positive bacteria, this resistance can be related to this thickness. There is also the possibility of lipophilic structure of antimicrobial compounds of lactic acid bacteria that due to the presence of lipid compounds such as lipoprotein, lipopolysaccharide and phospholipid in the cell wall of the gram-negative bacteria and in the outer membrane, the permeability of the cell wall to these antimicrobial metabolites probable Lipophilia is justifies (Shihavy et al., 2010).

Since antibacterial activity of different isolates against gastrointestinal pathogen bacteria has been different, this activity can be attributed to production of bacteriocins and specific protein compounds rather than to acid Organic compounds produced by them. Because if this antibacterial activity is due to the production of organic acids produced by lactic acid bacteria, the same inhibitory activity should be observed against all pathogenic bacteria. According to studies that inhibitory effects of different strains of Lactobacillus even in the same species have reported variable, this is confirmed (Lara-Villoslada et al., 2007; Schneitz et al., 1993; Kaushik et al., 2009; Serrano-Nino et al., 2016).

But considering all of these, the antibacterial activity of the metabolites produced by the lactic acid bacteria can be tested under the influence of

the pathogen microorganism tested, the physical and chemical parameters tested and the environmental conditions (Abebe et al., 2013).

4. 2. Tolerance of acid condition and bile salts of lactic acid bacteria isolated from colostrum samples

As mentioned, probiotics are living microbes that are eaten with food and provide beneficial effects for the host. Therefore, probiotic bacteria should be able to replace in gastrointestinal tract and tolerate gastric acid and intestine bile salts.

In this study, the ability to tolerate the acidic conditions of lactic acid bacteria isolated at different pHs of 2, 3 and 4 was evaluated.

Since the concentration of bile salts at the beginning of the intestine 1% and in the intestine up to 0.3%, these two concentrations of oxgall (mixture of cholic acid and taurocholic acid) were used to investigate the resistance of isolates to bile salts.

Results of these tests showed that *L. plantarum* was able to grow tolerate pH of 3 and in the presence of 0.3 and 1% bile salt (Oxgall).

Tolerance of *L. plantarum* to acidic conditions and high concentrations of bile salts has been reported in similar studies (Haghshenas et al., 2015; Pan et al., 2009; Abriouel et al., 2012).

Lactic acid bacteria from *Lactobacillus* and *Leuconostoc* genres are capable of producing an enzyme called Hydrolase Bile salt (HBS) that catalyzes bile salts to glycine and turin and allows bacteria to survive in the presence of bile salts in the gastrointestinal tract (Argyri et al., 2013; Lara Mantilla and Burgos Portacio, 2012; Schillinger et al., 2005).

The tolerance of some lactic acid bacteria to acidic pH is related to the production of the enzyme ATPase, which is found in this group of bacteria (Lertworapreecha et al., 2011).

Generally, considering the significant antibacterial activity of *L. plantarum* against all ten gram-positive and gram-negative bacteria tested and the ability to grow in acidic condition and various concentrations of bile salts, the probiotic potential of this isolate was evaluated as desirable.

Like of the present study, in research of Tambekar and Bhutada (2010) *L. plantarum* isolated from goat and cow's milk were able to grow at an acidic pH of 2 and at a concentration

of 2% bile salts. Also their antagonistic effect proved against gram-negative bacteria (Tambekar and Bhutada, 2010).

Dubos et al. (2011) also reported tolerance to acidic conditions and bile salts in *L. plantarum*, *L. fermentum* and *L. pentosus* and these isolates were nominated as probiotic candidates (Dubos et al., 2011).

In a similar study by Haghshenas et al. (2015) Two isolates of *L. plantarum* isolated from colostrum showed excellent antimicrobial effects, resistance to acidic pH and high concentration of bile salts, so the candidates were used as a probiotic (Haghshenas et al., 2015).

Considering all the studies, colostrum microbial composition and population included several factors including nutritional habits, milk compounds (vitamins, proteins, fatty acids, salts, etc.), genetics, intestinal microbial flora, livestock health, livestock weight, milk transfer type, use of antibiotics, age of gestational and lactation, and association with the skin (Fernandez et al., 2013; McGuire and McGuire, 2015; Reis et al., 2016).

Conclusion

In general, and according to the results of this study, colostrum as a traditional dairy food can be considered as a very suitable reservoir for the isolation of lactic acid bacteria with probiotic potential. Considering the significant antibacterial activity of *L. plantarum* against all ten gram-positive and gram-negative bacteria tested and the ability to grow in acidic condition and various concentrations of bile salts, the probiotic potential of this isolate was evaluated as desirable. Of course, more extensive tests are recommended to identify effective compounds and tolerate gastrointestinal conditions in *in vivo* conditions.

Author Contributions

H.K designed the study and interpreted the results and drafted the manuscript. Z.N collected test data. H.P consulted in research design and data analysis.

Conflicts of interest

There was no conflict of interest in this study.

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