Biological Journal of Microorganism
4th Year, Vol. 4, No. 16, Winter 2016
Received: April 28, 2015/ **Accepted:** October 28, 2015. **Page:** 97- 108

Isolation of a potentially probiotic *Lactobacillus plantarum* from Siahmezgi cheese and its characterization as a potentially probiotic

Hojjatolah Zamani *
Assistant professor of Microbiology, Department of Biology, University of Guilan, Rasht, Iran, h_zamani@guilan.ac.ir

Abstract

Introduction: Probiotics are non- pathogen living microorganisms which express beneficial effects on host, when are administered in adequate amounts. Traditional dairy products are regarded as good sources of probiotics. Present study aims to isolate Lactic Acid Bacteria (LAB) from Siahmezgi cheese, a traditional cheese produced in the north of Iran, and to evaluate their probiotic potential for the first time.

Materials and methods: LAB was isolated by culturing cheese samples on MRS agar. The isolates were screened for their probiotic potential using *in-vitro* tests, including tolerance to acid and bile, survival in simulated gastrointestinal tract conditions, β- galactosidase and hemolytic activity as well as antibiotic susceptibility. In addition, antibacterial activity of the isolated strains against *E. coli* O_{157} and *Salmonella enterica* serovar *typhimurium* ATCC 14028 was determined.

Results: One strain, labeled as Lb_3 showed the highest tolerance to low pH, bile and simulated gastrointestinal tract conditions. This strain exhibited resistance to Streptomycin, Vancomycin and Polymixin B as well as effective antibacterial activity against two Gram negative pathogens, lacking hemolytic activity as well as high β- galactosidase activity. Finally, the strain Lb_3 was identified as *Lactobacillus plantarum* CJLP55 using biochemical characterization and *16S rRNA* sequencing assay.

Discussion and conclusion: In the present work, a potentially probiotic *Lactobacillus plantarum* CJLP55 was isolated from traditionally produced Siahmezgi cheese. The bacterium displayed good probiotic properties and could be used in dairy industry.

Key words: Lactic acid bacteria, *Lactobacillus plantarum*, Probiotics, Siahmezgi cheese

*Corresponding Author

Introduction

Probiotics are microbial food supplements that have a beneficial effect on the host by influencing the composition and/or metabolic activity of intestinal microflora (1). Many beneficial effects of these microorganisms including anti-inflammatory properties, modulation of host's immune responses, reduction of lactose intolerance as well as inhibition of pathogenic bacteria have been described (2 & 3). A suitable probiotic strain must tolerate acidic pH of stomach and bile salts of intestinal tract and be able to adhere to mucosal surfaces (4).

Two genera *Lactobacillus* and *Bifidobacterium*, commonly present in dairy products are regarded as the major representative member of Lactic Acid Bacteria (LAB) and the most important probiotics (5). Traditional yogurts and cheese have been considered as good sources of LAB and ideal vehicles to deliver probiotic bacteria to human gastroinstestinal tract (6). Lotfi et al. (7) isolated 31 LAB from traditional cheese and yogurt from Heris and Sarab (East Azerbaijan, Iran) which belong to genus *Lactobacillus* and *Enterococcus*. In addition, Ghobadi Dana et al. (8) isolated a large number of LAB from traditional dairy products made from cow, sheep and goat milk and characterized their phylogenic relationship. Moreover, Islami et al. (9) isolated six *Enterococci* species from traditional cheese from Kaleibar (East Azerbaijan, Iran).

Siahmezgi cheese is a traditional dairy product which is produced using milk from domestic goats and sheep in highlands of Guilan province, Iran. The probiotic potential of LAB isolated from Siahmezgi cheese has never been investigated. Thus, aim of the present study was to isolate LAB from Siahmezgi cheese and to investigate their probiotic potential. In addition, inhibitory activity of the isolates against *E. coli* O_{157} and *Salmonella typhimurium* has also been studied.

Materials and methods

Sample collection and isolation of LAB: Samples were collected aseptically from ten different sites located at villages (that traditionally produce Sihmezgi cheese), province of Guilan, Iran, stored at 4°C and transferred immediately to the laboratory. The samples were mixed and homogenized in 0.85% sterile NaCl solution (1:10 w/v) on a shaker (600 rpm) for ten minutes. Homogenized samples were diluted serially and cultured on MRS agar (Merck GmbH, Germany). After incubation at 37°C for 48 h, the Gram positive, Catalase negative, non motile bacterial isolates were purified using streak culture technique and stored at 4°C.

Acid and bile tolerance: Tolerance to acid was determined according to the method described earlier (10), as the following: isolated strains were grown in MRS broth at 37ºC for 24-48 h, and sub-cultured in fresh MRS broth adjusted to pH 2.0 and 3.0 with hydrochloric acid (3.0 mol/L). The initial bacterial concentration was adjusted to 10^9cfu/mL and the survival rate was determined after incubation for 60 and 120 min at pH 2.0 and pH 3.0 which

reflects the time spent by food in the stomach (11). Survival rate was determined using plating on MRS agar and calculating viable counts at different intervals.

Bile tolerance test was conducted using the method described by Gilliland et al. (12). Overnight cultures of the isolated strains with initial concentration of 10^9cfu/mL were inoculated into MRS broth containing 0.5, 1.0 and 2.0 % (w/v) bile (Sigma-Aldrich). Survival rate of the isolates were measured following 24 h incubation at 37°C using the method described for acid tolerance assay.

Resistance to Lysozyme, Pepsin and Pancreatin: In order to evaluate resistance of Lb2 and Lb3 strains to Lysozyme, fresh bacterial cultures were pelleted by centrifugation, washed twice with PBS and re-suspended in 2 mL of Ringer solution (Sigma-Aldrich) to reach a final concentration of 10^9cfu/mL. In order to simulate in vivo dilution by saliva, the bacterial suspensions were inoculated in a sterile electrolyte solution (0.22 g/L $CaCl_2$, 6.2 g/L NaCl, 2.2 g/L KCl, 1.2 g/L $NaHCO_3$) in the presence of 100 mg/L of Lysozyme (Sigma-Aldrich). Bacterial suspensions without Lysozyme were also included as controls. Samples were incubated at 37°C and microbial survival rates were calculated as percentage of the cfu/mL after 0.5 and 2 h compared to the cfu/mL at time 0 (13).

Resistant of the isolated strains to Pepsin and Panceratin was also evaluated using the method described, previously (14). Briefly, fresh bacterial culture was harvested (6000 g, 10 min, 4°C), washed in Phosphate Buffer Saline (PBS) solution and re-suspended in PBS solution pH 2, containing 3 mg/mL Pepsin (Sigma) and PBS solution pH 8, containing 1 mg/mL Panceratin (Sigma) with the initial concentration of 10^9cfu/mL. After incubation at 37°C for 1 and 3 h with Pepsin, and 1 and 4 h with Panceratin, reflecting the time spent by food in the stomach and small intestine, respectively (11), the survival rate of the strains was determined.

Antibiotic susceptibility and hemolytic activity: Susceptibility of the isolated strains to the antibiotics commonly used by human was evaluated according to the method described previously (5). Briefly, the cultures were overlaid on Muller-Hinton agar plate and antibiotic discs were placed on it and incubated 24 h at 37°C. The assay was performed in triplicates and mean diameter of inhibition zones around antibiotic discs were recorded. The susceptibility was expressed in terms of resistance (R), intermediate susceptibility (I), and susceptibility (S) based on data from the National Committee for Clinical Laboratory Standards (NCCLS).

In order to evaluate hemolytic activity of the isolates, fresh bacterial cultures were plated on Blood Agar plate containing 5% v/v human blood. The plates were examined for signs of β- hemolysis (clear zones around colonies), α- hemolysis (green-hued zones around colonies) or γ- hemolysis (no zones around colonies) following 24-48 h incubation at 37°C (15).

β- Galactosidase activity: β-Galactosidase activity of the isolates was

determined using the method described by Meira et al. (16) with minor modifications. Briefly, the isolates were cultivated in lactose-MRS broth. The cells were washed and incubated with o-nitrophenyl-β-D-galactopyranoside (ONPG; Sigma) at 37°C for 15 min. Then, absorbance values were measured at both 420 and 560 nm and β-galactosidase activity was calculated and expressed as Miller units using the following formula: where, A1 was the absorbance just before assay and A2 was the cell density of the reaction mixture.

$$\beta\text{- Gal activity} = 1000 \times [(A_{420} - 1.75 \times A2_{560}] / (15\text{min} \times 1\text{ mL} \times A1_{560})$$

Antimicrobial activity against Gram negative pathogens: Antimicrobial activity of the isolated strains against *E. coli* O_{157} and *S. enterica* serovar *Typhimurium ATCC 14028* (hereafter refereed ad *S. typhimurium*) was investigated using the method described, previously (10 & 17). Microbial pathogens were provided by the Iranian Research Organization for Science and Technology (IROST), Tehran, Iran. A fresh culture of the isolated bacterium, grown in MRS broth, was centrifuged (6000 g, 10 min, 4°C) and the resulting supernatant was adjusted to pH 6.5 with NaOH (1 M) in order to rule out acid inhibition. The supernatant was used to determine antibacterial activity of the isolated strain against *E. coli* O_{157} and *S. typhiurium* using well diffusion assay. This assay was performed in triplicates and mean diameter of inhibition halos were measured.

Biochemical and molecular identification of the isolated strain: Based on the results of the assays described above, only one strain (isolate Lb_3) was identified using physiological and biochemical tests according to Bergey's Manual of Systematic Bacteriology (18). These included the study of growth at different temperatures (10, 37, 45 and 55ºC) and NaCl concentrations (2.5, 6.5 and 10 and 18%), determination of fermentation type, triple sugar utilization test, citrate utilization test as well as sugar utilization test. In order to confirm the result from biochemical characterization, *16S rRNA* sequencing was performed. Briefly, Total DNA from isolated strain was extracted using Bioneer genomic extraction kit according to the manufacturer's manual. Then, *16S rRNA* gene was amplified using the Hal F (5' AGAGTTTGATCCTGGCTCAG 3') and Lac R (5' AAGGTTACCTCACCGACTTC 3') primers (7). The thermal cycler was programmed as follows: 10 min at 94ºC; 30 cycles of 1 min at 94ºC, 1 min at 57ºC, 1 min at 72ºC and 5 min at 72ºC. The PCR product was sequenced for *16S rRNA* (GATC Biotech, Germany) and the sequence was submitted to Gen Bank (NCBI).

Statistical analysis: Assays were conducted in triplicates and statistical analysis was performed using SPSS version 18. The comparisons of differences between the means of the treatments were tested by *one way ANOVA* and a P value of less than 0.05 was considered significant.

Results

Isolation and identification of potent probiotic strain: Only six bacterial strains were isolated from Siahmezgi cheese which were selected for further studies based on the morphology, Gram staining, Catalase production and motility tests. Only two Gram positive, Catalase negative, non motile rods (isolates Lb_2 and Lb_3) were regarded as LAB and further characterized as potent probiotic strains and other isolates were discarded.

According to the results, only one strain (Lb_3) was found to display potentially probiotic characteristics. Identification of this strain using physiological, biochemical and molecular assays was performed. The growth was observed at 10, 37 and 45ºC but the strain did not tolerate 55 ºC. In addition, the culture grew well in the media containing 2.5, 6.5 and 10% salt but did not grow in the medium containing 18% NaCl. Table 1 displays the results from determination of fermentation type, triple sugar utilization test (TSI slant) and citrate utilization tests.

Table1- Some growth characteristics of the isolated LAB

Characteristics parameter	Lb_2	Lb_3
Growth temperature (°C)		
10	+	+
37	+	+
45	-	+
Growth in NaCl (%)		
2.5	+	+
6.5	+	+
10	-	+
18	-	-
Fermentation type	MR (mixed acid)	MR(mixed acid)
TSI slant	A/A, H_2S^-, gas^+	A/A, H_2S^-, gas^-
Citrate utilization	-	-

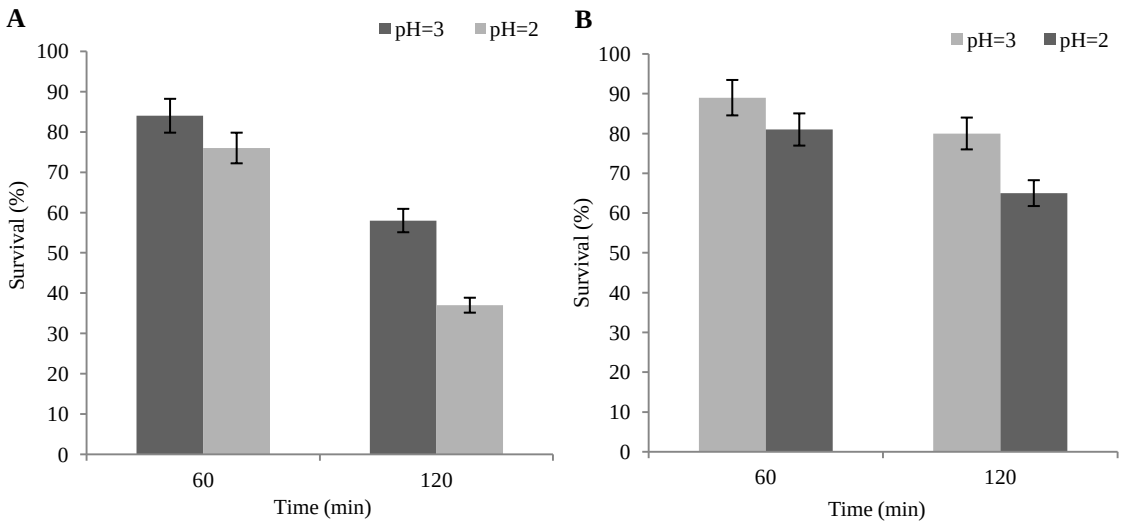

Fig. 1- Tolerance of isolated strains to low pH a) Lb_2 and b) Lb_3

According to the results from biochemical assays, the isolate Lb_3 was tentatively identified as *Lactobacillus plantarum*. The isolate Lb_3 was subjected to *16S rRNA* sequencing and the cultures were found to have 99% sequence similarity to *L. plantarum* CJLP55, confirming biochemical characterization assays.

Acid and bile tolerance: Acid tolerance assay showed that both Strains Lb_2 and Lb_3 tolerated pH 3 after 120 min with survival percentage of 58 and 80% respectively, but the strain Lb_3 displayed significantly higher tolerance to 120min exposure to pH 2 with survival rate of 65%, compared to strain Lb_2 with survival rate of 37%. Fig. 1 displays the results of acid tolerance of the bacterial isolates at pH 3.0 and 2.0 studied for a period of 60 and 120 min.

Bacterial isolates were treated with different concentrations of bile salts. According to the results, both strains showed decrease in viability by increasing bile concentration. The isolate Lb_2 showed only 55, 37 and 22% tolerance following exposure to 0.5, 1.0 and 2% bile concentrations, respectively. Conversely, isolate Lb_3 showed significantly higher tolerance to similar concentrations of bile with 73, 68 and 60% viability, respectively (Fig. 2).

Resistance to condition simulating GI tract: The overall resistance of the isolates to Lysozyme was determined and expressed as survival rates. According to the result, the isolates Lb_2 and Lb_3 showed a high Lysozyme resistance with survival rates of 76 and 83% respectively, following 30 min exposure to 100 mg/L Lysozyme. Table 2 reports data of bacterial survival after treatment with Lysozyme for 30 and 120 min.

Our results revealed that the isolate Lb_2 was highly susceptible to Pepsin solution (pH 2.0) while, the solution had less effect on survival rate of isolate Lb_3. Isolate Lb_2 showed survival percentage of 49 and 33% after exposure to Pepsin solution after one and three hours, while strain Lb_3 had a moderate decrease in the number of survivors at similar condition with survival rate of 57 and 48% following one and three hours exposure to Pepsin solution. In contrast, exposure to Pancreatin containing solution (pH 8.0) did not dramatically decrease survivors of both strains even after 4h incubation (Table 2).

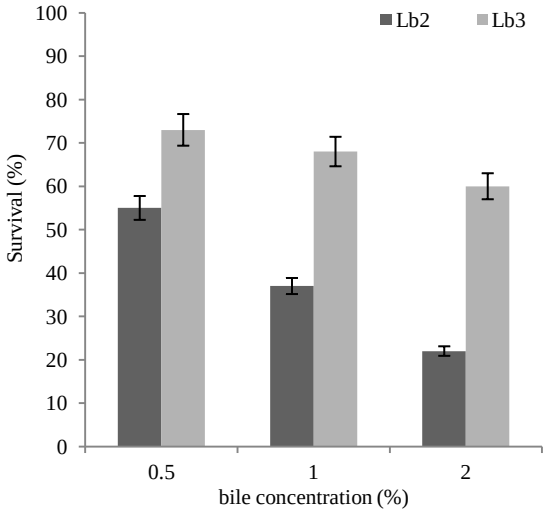

Fig 2- Effect of bile concentration on survival rate of the isolated strains following 24h incubation

Table 2- Survival rate of isolated strains in simulated gastrointestinal condition

	Survival rate in simulated saliva condition (Lysozyme) (%)		Survival rate in simulated gastric condition (Pepsin solution, pH 2.0 (%)		Survival rate in simulated intestinal condition (Panceratin solution, pH 8.0) (%)	
Isolates / Time	0.5h	2h	1h	3h	1h	4h
Lb_2	76	64	49	33	75	73
Lb_3	83	76	57	48	72	70

Table 3- Susceptibility of the isolated strains to different antibiotics

	Concentration	Zone inhibition (mm)		Response	
Antibiotics		Lb_2	Lb_3	Lb_2	Lb_3
Erythromycin	15 µg	28	34	S	S
Streptomycin	10µg	5	5	R	R
Kanamycin	30µg	11	16	R	I
Vancomycin	30µg	13	10	R	R
Penicillin	10 IU	29	34	S	S
Gentamicin	10µg	14	19	I	S
Polymyxin B	300 IU	8	7	R	R

R: Resistance, I: Intermediate susceptibility, S: Susceptibility

Antibiotic susceptibility and hemolytic activity: Isolate Lb_2 showed resistance to majority of antibiotics used in this study, but susceptibility to erythromycin and penicillin was observed. In contrast, *L. plantarum* CJLP55 isolated in this study was able to resist Vancomycin, Polymyxin B as well as streptomycin. Table 3 compares antibiotic resistance of both isolates. In addition, none of isolates exhibited hemolytic activity.

β– galactosidase activity: In this study, β–galactosidase activity of the isolates was determined. Both isolates showed high level of β–galactosidase activity with average values of 539.57 and 472.44 Miller units for the isolates Lb_2 and Lb_3 respectively.

Antimicrobial activity against Gram negative pathogens: Antibacterial activity is an important feature of the probiotic strains. The isolates were checked for their antibacterial activity against two gastrointestinal pathogens, *E. coli* O_{157} and *S. typhimurium*. Our results showed that both isolates inhibit indicator pathogen bacteria with different inhibition level. Isolate Lb_2 was weakly effective against *E. coli* O_{157} with inhibition zone diameter of 2 mm and slightly more effective against *S. typhimurium* with inhibition zone diameter of 5 mm, whereas evaluation of antibacterial activity of the isolate Lb_3 (*L. plantarum*) showed stronger antibacterial activity against *E. coli* O_{157} and *S. typhimurium* with inhibition zone diameter of 7 And 11 mm respectively.

Discussion and conclusion

In the current work, Siahmezgi cheese was examined in order to isolate LAB and some established *in-vitro* tests were applied to evaluate the probiotic potential of the isolates. Only few microorganisms survive in cheeses because of its low redox, low pH and high salt (19- 20). Among the isolates only one strain (Lb3) was found to display potentially probiotic characteristics.

Identification of this strain using biochemical and molecular assays showed the presence of *L. plantarum* CJLP55 as a potent probiotic strain in Siahmezgi cheese. Isolation of *L. plantarum* strains from dairy products including traditional cheese has been reported previously (13 & 21). Lotfi et al. (7) isolated *L. plantarum* CJLP55 from traditional dairy products of north-west of Iran. They reported that only few species tolerate low pH and high bile salts which were identified as *L. plantarum*, *L. casei*, *E. hirae* and *E. durans*. A large number of *L. plantarum* strains were reported to possess potential probiotic properties with health-promoting effects (13 & 22).

For a probiotic strain, survival under gastrointestinal environment condition is important criteria to be fulfilled which depends on tolerance to low pH and high bile concentration as well as resistance to gastrointestinal enzymes. Tolerance to extremely acidic condition is an important feature of a probiotic strain (23). *L. plantarum* CJLP55 isolated from Siahmezgi cheese showed good tolerance to low pH showing its ability to survive under acidic environment of stomach. This strain was more tolerant to low pH in comparison with the *L. plantarum* studied by Lotfi et al. (7) who isolated LAB from traditional cheese from Heris and Sarab regions. Many researchers attributed the acid tolerant nature of LAB to induction of H^+- ATPase activity (24- 25). Thus, higher tolerance isolate Lb_3 compared to isolate Lb_2 might be attributed to higher activity of H^+-ATPase.

The physiological concentration of bile in the small intestine has been reported to be between 0.2 and 2.0% (26*). L. plantarum* CJLP55 isolated in our study showed a good tolerance to 2.0% bile with survival rate of 60%. The good bile tolerance of the isolated *L. plantarum* has been reported previously (27). Similar results were also reported by Mourad and Nour-Eddine (28) who found that one of their *L. plantarum* isolates showed 65% survival rate following exposure to 2.0% bile. Resistance to bile salts varies a lot among lactic acid bacteria species and even between strains themselves. Bile resistance of some strains is related to the specific enzymatic activity of Bile Salt Hydrolase (BSH) which helps hydrolyzing conjugated bile, thus reducing its toxic effect (29). BSH activity is a widespread trait in *L. plantarum* (30). In a study, Zago et al. (13) reported that all their *L. plantarum* isolates had bsh gene and expressed BSH activity.

Resistance to Lysozyme is another feature of probiotic strains. The strain isolated in our study revealed a good resistance to Lysozyme, confirming previous report (13). Resistance to Lysozyme has been attributed to the peptidoglycan structure in the cell wall, physiological state of the cell and Lysozyme structure in the medium (30-31).

Frequent antibiotic administration causes gut microbiota imbalance and an increased susceptibility to infection was caused by opportunistic microorganisms (32). Probiotic strains which are resistant to antibiotics can proliferate in gut and maintain microbial balance and reduce

opportunistic microorganisms (5). According to the results, the isolated *L. plantarum* strain is able to resist Vancomycin, Polymyxin B as well as Streptomycin. Similar antibiotic resistance pattern of probiotic *L. plantarum* strains was reported by Yu et al. (10), but unlike their finding, the strain isolated in this study was susceptible to Gentamycin. The ability of probiotics to resist antibiotics might be beneficial to people suffering from intestinal disorders due to improper administration of antibiotics (33). However, it is important that the bacterial strains involved do not transfer antibiotic resistance genes from foods to intestinal microflora (34). Absence of hemolytic activity is another considered safety prerequisite for the selection of a probiotic strain. None of isolates exhibited hemolytic activity, confirm their safety as potent probiotic strains.

β–galactosidase, an enzyme responsible for the hydrolysis of lactose into simple sugars, is widely used in dairy industry in order to prepare lactose-free dairy products. In addition, lactose intolerance is found in people lacking the enzyme β -galactosidase, since lactose is not broken down in the upper regions of the small intestine and is thus used by the indigenous microbiota (35). The strain isolated in our study displayed high level β –galactosidase activity. Thus, it could be suggested that this strain could be explored in lactose rich dairy products to increase lactose tolerance in lactose-intolerant individuals.

Antibacterial activity is an important feature of the probiotic strains. Isolated *L. plantarum* CJLP55 was evaluated for its inhibitory activity against two food-borne pathogens, *E. coli O157 and S. typhimurium*. *L. plantarum* revealed a good antimicrobial activity against indicator microorganisms. Antimicrobial activity of *L. plantarum* against potential pathogens has been reported by Yu et al. (10), while some studies did not show effective antibacterial activity of *L. plantarum* strains against pathogenic bacteria (11). This property of the isolated bacterium can be used in prophylactic or therapeutic usage. The inhibitory activity of the *L. plantarum* strains might be due to either production of organic acids or bacteriocines (10). Since the supernatant used in this study was adjusted to neutral pH, the antibacterial activity might be attributable to bacteriocins produced by *L. plantarum*.

In this study, Siahmezgi cheese was examined to isolate potentially probiotic bacteria for the first time and a *L. plantarum* with good probiotic properties was isolated. However, very few strains isolated in this study restricted our chance for selecting more proper strains which could be attributed to the aerobic culture condition. Thus, further investigations including anaerobic and microaerophilic culture conditions could be employed to isolate other potentially probiotic strains from siahmezgi cheese.

L. plantarum CJLP55 as a potential probiotic strain was isolated from Siahmezgi cheese for the first time and its probiotic features were characterized. This isolate showed tolerance to high bile concentration, low pH and survived under

condition simulating human gastrointestinal tract. Thus, it could be predicted that the isolate would be able to pass stomach and reach intestine in adequate amounts. In addition, this strain displayed a good antibacterial activity against two Gram negative food-borne pathogens. Thus, *L. plantarum* CJLP55 could be considered as a good probiotic candidate. However, further investigations including in-vivo experiments as well as molecular analysis would be helpful to elucidate its potential health benefits and application as a probiotic strain in the dairy industry.

References

(1) Mourad K., Nour-Eddine K. In vitro preselection criteria for probiotic Lactobacillus *plantarum* strains of fermented olives origin. *International Journal of Probiotics and Prebiotics* 2006; 1 (1): 27- 32.

(2) De Vrese M., Schrezenmeir J. Probiotics, prebiotics, and synbiotics. Book section In: Food Biotechnology. Advances in Biochemical Engineering/Biotechnology, vol. 111. Berlin: Stahl, U., Donalies, U. E. B., Nevoigt, E. (Eds.); Springer- Verlag, pp. 1-66. 2008.

(3) Leahy SC., Higgins DG., Fitzgerald GF., Van Sinderen D. Getting better with *Bifidobacteria*. *Journal of Applied Microbiology* 2005; 98 (6): 1303- 15.

(4) Shobharani P., Agrawal R. A Potent Probiotic Strain from Cheddar Cheese. *Indian Journal of Microbiology* 2011; 51(3): 251- 8.

(5) Le Blanc A M., Castillo NA., Perdigon G. Anti-infective mechanisms induced by a probiotic Lactobacillus strain against *Salmonella enterica* serovar *Typhimurium* infection. *International Journal of Food Microbiology* 2010; 138 (3): 223- 31.

(6) Karimi R., Mortazavian AM., Amiri-Rigi A. Selective enumeration of probiotic microorganisms in cheese. *Food Microbiology* 2012; 29 (1): 1- 9.

(7) Lotfi H., Hejazi MA., Maleki Zanjani B., Barzegari A. Isolation, Biochemical and Molecular Identification of Potentiallly Probiotic Bacteria from Traditional Dairy Products from Heris and Sarab Regions. *Journal of Food Research* 2010; 20/3 (1): 1- 17 (article in Persian).

(8) Ghobadi Dana M., Hatef Salmanian A., Yakhchali B. Isolation and identification of indigenous Lactobacilli in traditional dairy products in Iran. *Journal of research and innovation in food science and technology* 2011; 1 (2): 99- 116.

(9) Eslami S., Hejazi MA., Ebrahimipour G., Barzegari A. Identification of Enterococcus species isolated from traditionally produced cheese of kaleybar by sequencing and restriction enzyme digeston of 16S rDNA gene. Proceeding of the 1st natonal conference of probiotic and functional food; 2010 April 21-22; Tehran, Iran.

(10) Yu Z., Zhang X., Li S., Li C., Li D., Yang Z. Evaluation of probiotic properties of *Lactobacillus plantarum* strains isolated from Chinese sauerkraut. *World Journal of Microbial Biotechnology* 2013; 29 (3): 489- 98.

(11) Maragkoudakis PA., Zoumpopouloua G., Miarisa C., Kalantzopoulosa G., Potb B., Tsakalidou E. Probiotic potential of Lactobacillus strains isolated from dairy products. *International Dairy Journal* 2006; 16 (3): 189- 99.

(12) Gilliland SE., Staley TE., Bush LJ. Importance in bile tolerance of *Lactobacillus acidophilus* used as a dietary adjunct. *Journal of Dairy Science* 1984; 67 (12): 3045- 51.

(13) Zago M., Fornasari ME., Carminati D., Burns P., Suàrez V., Vinderola G., et al. Characterization and probiotic potential of *Lactobacillus plantarum* strains isolated from cheeses. *Food Microbiology* 2011 (5); 28: 1033- 40.

(14) Charteris WP., Kelly PM., Morelli L., Collins JK. Development and application of an in vitro methodology to determine the transit tolerance of potentially probiotic Lactobacillus and *Bifidobacterium* species in the upper human gastrointestinal tract. *Journal of Applied Microbiology* 1998; 84 (5): 759- 68.

(15) Zoumpopoulou G., Foligne B., Christodoulou K., Grangette C., Pot B., Tsakalidou E. *Lactobacillus fermentum* ACA-DC 179 displays probiotic potential in vitro and protects against trinitrobenzene sulfonic acid (TNBS)-induced colitis and Salmonella infection in murine models. *International Journal of Food*

Microbiology 2008; 121 (1): 18- 26.

(16) Meira SMM., Helfer VE., Velho RV., Lopes FC., Brandelli A. Probiotic potential of Lactobacillus spp. isolated from Brazilian regional ovine cheese. *Journal of Dairy Research* 2012; 79 (1): 119- 127.

(17) Ebrahimi Alavijeh S., Mahzounieh M., Mobini Dehkordi M. Effects of metabolites of Lactobacilli isolated from local yoghurts in Chahrmahal va Bakhtiary on pathogenic bacteria. *Biological Journal of Microorganism* 2013; 2 (5): 11- 20.

(18) Iyer BK., Singhal RS., Ananthanarayan L. Characterization and in vitro probiotic evaluation of lactic acid bacteria isolated from *idli* batter. *Journal of Food Science and Technology* 2011; 50 (6): 1114- 21.

(19) Peterson SD., Marshall RT., Heymann H. Peptidase profiling of Lactobacilli associated with cheddar cheese and its application to identification and selection of strain of cheese ripening studies. *Journal of Dairy Science* 1990; 73 (6): 1454- 64.

(20) Salminen S., Laino M., Von Wright A., Vuopio-Varkila J., Korhonon T., Mattila-Sandholm T. Development of selection criteria for probiotic strains to assess their potential in functional foods: a Nordic and European approach. *Bioscience Microflora* 1996; 15 (2): 61–67.

(21) Coeuret V., Gueguen M., Vernoux JP. In vitro screening of potentially probiotic activities of selected lactobacilli isolated from unpasteurized milk products for incorporation into soft cheese. *Journal of Dairy Research* 2004; 71 (4): 451- 60.

(22) Maaike C., de Vires E., Vaughan E., Kleerebezem M., de Vos WM. *Lactobacillus plantarum* survival, functional and potential probiotic properties in the human intestinal tract. *International Dairy Journal* 2006; 16 (9): 1018- 28.

(23) Guo Z., Wang J., Yan L., Chen W., Liu X., Zhang H. In vitro comparison of probiotic properties of *Lactobacillus casei* Zhang, a potential new probiotic, with selected probiotic strains. *LWT- Food Science and Technology* 2009; 42 (10): 1640- 6.

(24) Matsumoto M., Ohishi H., Benno Y. H+-ATPase activity in *Bifidobacterium* with special reference to acid tolerance. *International Journal of Food Microbiology* 2009; 93 (1): 109- 13.

(25) Ventura MD., Van Sinderen G., Fitzgerald F., Zink R. Insights into the taxonomy, genetics and physiology of *Bifidobacteria*. *Antonie van Leeuwenhoek* 2004; 86 (3): 205- 33.

(26) Gunn JS. Mechanisms of bacterial resistance and response to bile. *Microbes Infection* 2000; 2 (8): 907- 13.

(27) García-Ruiz A., de Llano DG., Esteban-Fernández A., Requena T., Bartolomé B., Moreno-Arribas MV. Assessment of probiotic properties in lactic acid bacteria isolated from wine. *Food microbiology* 2014; 44, 220- 5.

(28) Mourad K and Nour-Eddin K. In Vitro preselection criteria for probiotic Lactobacillus plantrum strains of fermented olives origin. *International Journal of Probiotics and Prebiotics* 2006; 1 (1): 27- 32.

(29) Du Toit M., Franz C., Schillinger U., Warles B., Holzappfel W. Characterization and selection of probiotic lactobacilli for a preliminary minipig-feeding trail and their effect on serum cholesterol level, faeces moisture contents. *International Journal of Food Microbiology* 1998; 40: 93- 104**.**

(30) Pisano MB., Casula M., Corda A., Fadda ME., Deplano M., Cosentino S. In vitro probiotic characteristics of Lactobacillus strains isolated from Fiore Sardo cheese. *Italian journal of food science* 2008; 20 (4): 505- 16.

(31) Cunningham FE., Proctor VA., Goetsch SJ. Egg-white lysozyme as a food preservative: an overview. *World's Poultry Science Journl* 1991; 47(2): 141-163.

(32) Willing BP., Russell SL., Finlay BB. Shifting the balance: antibiotic effects on host–microbiota mutualism. *Nature Reviews Microbiology* 2011; 9: 233- 43.

(33) Salminen S., Von Wright A., Morelli L., Marteau P., Brassart D., de Vos WM., et al. Demonstration of safety of probiotic: a review. *International Journal of Food Microbiology* 1998; 44 (1- 2): 96- 106.

(34) Mathur S., Singh R. Antibiotic resistance in food lactic acid bacteria-a review. *International Journal of Food Microbiology* 2005; 105 (3): 281- 95.

(35) De Vrese M., Stegelmann A., Richter B., Fenselau S., Laue C., Chrezenmeie J. Probiotics compensation of lactase insufficiency. *American Journal of Clinical Nutrition* 2001; 73 (2): 421- 9.

جداسازی باکتری *Lactobacillus plantarum* از پنیر سیاهمزگی و تعیین ویژگی‌های پروبیوتیکی آن

حجت الله زمانی [*]: استادیار میکروبیولوژی، گروه زیست شناسی، دانشگاه گیلان، رشت، ایران، h_zamani@guilan.ac.ir

چکیده

مقدمه: پروبیوتیک‌ها میکروارگانیسم‌های زنده و غیر بیماری‌زایی هستند که اگر به تعداد کافی وارد بدن شوند، خواص سلامت بخش زیادی را برای میزبان به ارمغان می‌آورند. محصولات لبنی سنتی منبع مناسبی برای جداسازی میکروارگانیسم‌های با خواص پروبیوتیکی به شمار می‌آیند. هدف از این پژوهش، جداسازی برخی از باکتری‌های اسید لاکتیک از پنیر سیاهمزگی (نوعی پنیر سنتی شمال ایران) و بررسی ویژگی‌های پروبیوتیکی باکتری جداشده برای نخستین بار است.

مواد و روش‌ها: باکتری‌های اسید لاکتیک به دنبال کشت نمونه‌های پنیر سیاهمزگی روی محیط کشت MRS agar جداسازی شدند. باکتری‌های جداسازی شده در شرایط آزمایشگاهی از نظر ویژگی‌هایی همچون میزان مقاومت به اسید و صفرا، بقا در شرایط رشدی مشابه دستگاه گوارش، ویژگی‌های همولیتیک، تولید آنزیم بتاگالاکتوزیداز و الگوی حساسیت آنتی‌بیوتیکی ارزیابی شدند. علاوه بر این، ویژگی‌های ضد میکروبی باکتری‌های جداسازی شده بر علیه باکتری‌های *اشرشیاکلی* O_{157} و *سالمونلا انتریکا سروار تایفی موریوم* (ATCC 14028) بررسی شد.

نتایج: در پایان، آزمایشات یک سویه (Lb_3) بهترین ویژگی‌های مقاومتی به شرایط مشابه دستگاه گوارش را نشان داد. همچنین، این باکتری نسبت به آنتی‌بیوتیک‌های ونکومایسین و پلی میکسین B مقاومت کرده در حالی که فعالیت ضد میکروبی مناسبی بر علیه سویه‌های پاتوژن مورد آزمایش نشان داد. در نهایت، این باکتری با به‌کارگیری روش‌های شناسایی بیوشیمیایی و توالی یابی *16S rRNA* به عنوان *Lactobacillus plantarum* CJLP55 شناسایی شد.

بحث و نتیجه‌گیری: در این مطالعه، باکتری *Lactobacillus plantarum* از پنیر سنتی سیاهمزگی جداسازی شد و به عنوان یک باکتری دارای ویژگی‌های پروبیوتیک معرفی شد که می‌تواند در صنایع لبنی استفاده شود.

واژه‌های کلیدی: پروبیوتیک، پنیر سیاهمزگی، باکتری‌های اسیدلاکتیک، *لاکتوباسیلوس پلانتاروم*

---

* نویسنده مسؤول مکاتبات
تاریخ دریافت: ۱۳۹۴/۰۲/۰۸- تاریخ پذیرش: ۱۳۹۴/۰۸/۰۶