Hittite Journal of Science and Engineering, 2015, 2 (2) 181-185
ISSN NUMBER: 2149-2123
DOI: 10.17350/HJSE19030000023

Determination of Fundamental Probiotic Properties of *Lactobacillus* Strains Isolated from Turkish Local Yogurt

Gulcin Alp Avci[1], Emre Avci[1], Asiye Aslı Emniyet[2] and Burcin Ozcelik[3]
[1] Hitit University, Department of Molecular Biology and Genetics, Corum, TURKEY
[2] Hitit University, Department of Biology, Corum, TURKEY
[3] Hitit University, Department of Biology, Corum, TURKEY

ABSTRACT

Lactic acid bacteria have benefits for the digestive tract. They and some of their methabolic products stimulate the immune system and therefore regarded as probiotic. A good quality probiotic must have some features like stomach acid resistance, bile reduction, exopolysaccharide production and must be able to keep the intestinal mucosa. This research was aimed to determinate the resistance to acid, tolerance to bile and exopolysaccharide (EPS) production capacity of all *Lactobacillus* strains isolated from home-made yogurt gathered from 17 different Turkish villages. All strains were researched for acid and bile tolerance and EPS production. All values were measured spectrophotometrically. Acid and bile tolerance levels of isolates were in a different rate. And also, the lowest and highest EPS amounts were calculated as 20.82 and 121.01 mg/L, respectively. These features can be reference points to choose a probiotic microorganism.

Article History:
Received: 2015/11/10
Accepted: 2015/11/23
Online: 2015/12/30

***Correspondence to**: Gulcin ALP AVCI, Hitit University, Faculty of Science and Arts, Department of Molecular Biology and Genetics, Corum, TURKEY*
Tel: +90 (364) 2277000/1609
Fax: +90 (364) 2277005
E-Mail: gulcinalp@hitit.edu.tr, alp.gulcin@yahoo.com

Key Words:
Probiotic; *Lactobacillus*; Acid and Bile Tolerance; Exopolysaccharide Production.

INTRODUCTION

Lactic acid bacteria (LAB) convert carbohydrate to lactic acid and another product. They are gram-positive, aero-tolerant, catalase negative microorganisms and they do not include spore in their cell. They protect foods with fermentation from the spoilage and give a different taste and texture them. Very different fermented foods can be produced by using LAB like yogurt, butter, cheese, sausage and cereals products include probiotics [1-3]. LAB have a 'Generally Recognized as Safe' (GRAS) classification because of exopolysaccharides (extracellular polysaccharides, EPS) production specialty. EPS are metabolites located extracellular surface and emerge from the milk fermentation of LAB [4,5]. EPS production, because of its useful health effects, ensures functionality to the foods which include LAB [6]. The genera of *Lactobacillus, Streptococcus* and *Lactococcus* are EPS-producing lactic acid bacteria [7]. In general, LAB ferments sugars, produce lactic acid and can biosynthesis EPSs from this fermentation reaction [8]. LAB fermentation uses not only sugars but also some kind of fats, proteins, and organic acids which improve the food's typical aroma and texture. Milk was exposed to rapid acidification and lactic fermentation that prohibit proliferation of pathogens. Fermented milk products include EPS such as yogurt and cheese. In order to stay alive and be able to settle down the gastrointestinal area of LAB should express tolerance to acid and bile [9]. Lactic acid bacteria which are taken at diet should withstand stomach acid (pH 2) to reach intestine and to show probiotic effects. So a lactic acid bacteria strain must be resistant for acid and bile salt concentration [1, 10]

In this research, we aimed to determine acid and bile tolerance and EPS production capacity of *Lactobacillus* strains isolated from Turkish home-made yogurt.

MATERIAL & METHODS

Yogurt samples and isolation of *Lactobacillus* strains

Yogurt samples were collected from seventeen different villages in Corum/Turkey. Each sample diluted with phosphate saline buffer (PBS) from 10^{-1} to 10^{-6}. After dilution, lactic acid bacteria were isolated from Turkish local yogurt using MRS (**De Man, Rogosa and Sharpe**) broth and MRS agar. Diluted samples inoculated on MRS agar plates and they were incubated in 37°C. After incubation, single colonies were examined with a microscope and lactic acid bacteria were chosen by using gram staining and microscopical morphology. Cultures were kept in -86°C until experiment.

Determination of acid and bile tolerance

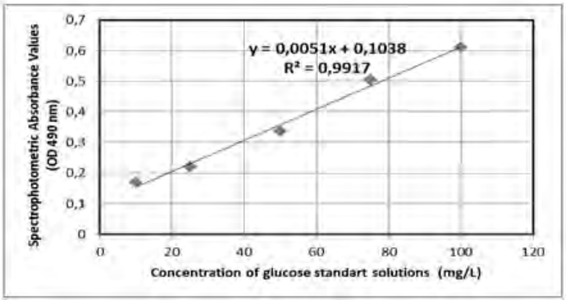
To determine the ability to reach the intestine through the stomach's highly acidic environment of *Lactobacillus* sp., the stomach environment was simulated. For this purpose, MRS Broth pH was adjusted as pH 3.0, 4.0, 5.0, 7.0, 8.0 and 6.2 (control) using 1 N HCl acid before sterilization. The optical density of isolates was measured of 600 nm. These isolates inoculated to the pH adjusted medium in 1% ration. After inoculation, cultures were incubated at 37±1°C temperature for 16-18 hours. When incubation was completed, optical density was measured spectrophotometrically in 600 nm wavelength.

To determine the bile tolerance a similar method to the acid resistance of bacteria was used. MRS broth was sterilized. After sterilization, bile salt was added to broth by sterilization with micro-filter in different rates (0.15%, 0.2%, 0.3 and 0.4 %, respectively). The optical density of isolates was measured of 600 nm. These isolates inoculated to the pH adjusted medium in 1% ration. After inoculation, cultures were incubated at 37±1°C temperature for 16-18 hours. When incubation was completed, optical density was measured spectrophotometrically in 600 nm wavelength.

Determination of EPS production capacity

EPS production capacity was studied using the phenol-sulfuric acid method modified by Alp [10]. Firstly, to release exopolysaccharide, lactic acid bacteria were exposed to some processes. 1 ml active culture (Optical Density ≈ 600) were put in Eppendorf tube and boiled in 96°C for 10-15 minutes. Then samples were cooled until room temperature and 1 ml trichloracetic acid (TCA) was added and centrifuged at 13000 rpm for 25 minutes. After this process, 1 ml ethyl alcohol added in the tube and centrifuged again. When centrifugation was finished, pellets could be obtained on the bottom of the tubes. The pellet was separated from the supernatant, solved in 1 ml ethyl alcohol and precipitated with centrifugation. Finally precipitated sample was separated from alcohol and solved 1 ml distilled water. Sample became ready to be used for the phenol-sulfuric acid method.

According to the phenol sulfuric acid method, 0.5 ml phenol and 5 ml sulfuric acid were added above the 1 ml samples in the tube. The tube was incubated in room temperature for 10 minutes and when the time up samples were mixed well. After stirring, the tube was incubated in 30°C for 15-20 minutes. When incubation was complete, optical density (OD) was measured spectrophotometrically at 490 nm wavelength.



Graphic 1. Calibration line which was drawn with glucose standart solutions and its equation

Statistical Analysis

All experiment was done in triplicate. The results were determined to standard curve. Statistical analyzes were performed on the data by IBM SPSS 22.0 and standard curve graphic was drawn using Microsoft Office Excel program.

RESULTS AND DISCUSSION

Determination of acid and bile tolerance

Probiotic microorganisms should resist the stressful conditions of the stomach and intestine [9]. Acid and bile are believed to be the most detrimental factor affecting growth and viability of lactobacillia [11, 12]. Therefore, these strains may be expected to stay alive in acid and bile conditions that exist in fermented food products or gastrointestinal area [13]. The acid tolerance of the lactobacillus strains isolated from the Turkish local yogurt was determined using different pH values. The results determined by the spectrophotometric method shown in Table 1. When we look at the results, stomach acid resistance was found as quietly low. As acid ratio increased, bacterial density was decreased in the medium. When we considered all the strains, bacterial density was decreased at pH 3. And also, bile tolerance of the lactobacillus strains was determined very similar to acid tolerance experiment. Tolerance to the bile in the intestine by *Lactobacillus* strains varied according to the bile salt rate. Although the concentration of the bile salt 0.15 % has triggered the growth of bacteria, media were including bile salt in rate 0.2%, 0.3% and 0.4% inhibited the development of all strains in general. Bile tolerance data illustrated in Table 2. In literature, some *Lactobacillus*

Table 1. Results of acid resistance in *Lactobacillus* strains isolated from Turkish local yogurt.

Strains	Control[b] (pH 6.2)	pH[a]				
		3.0	4.0	5.0	7.0	8.0
Strain 1	1.40±0.00	0.13±0.01	0.59±0.01	1.39±0.04	1.22±0.20	0.80±0.02
Strain 2	1.39±0.01	0.16±0.02	0.52±0.08	1.40±0.07	1.19±0.06	0.91±0.11
Strain 3	1.52±0.28	0.14±0.00	0.52±0.00	0.89±0.01	1.02±0.01	0.92±0.08
Strain 4	1.72±0.00	0.15±0.00	0.68±0.01	1.51±0.23	0.99±0.02	0.81±0.01
Strain 5	1.58±0.15	1.13±0.00	0.19±0.00	1.53±0.03	1.51±0.00	1.89±0.02
Strain 6	1.42±0.01	0.36±0.02	0.64±0.02	1.34±0.06	1.05±0.05	0.86±0.08
Strain 7	1.47±0.10	0.13±0.00	0.19±0.00	1.14±0.14	0.42±0.05	0.36±0.01
Strain 8	0.98±0.00	0.16±0.00	0.26±0.01	0.96±0.03	0.66±0.03	0.69±0.01
Strain 9	1.61±0.02	0.14±0.00	0.11±0.00	0.20±0.00	0.30±0.00	0.35±0.03
Strain 10	0.82±0.03	0.26±0.00	0.30±0.00	0.99±0.41	0.61±0.05	0.71±0.06
Strain 11	1.32±0.00	0.23±0.00	0.36±0.00	0.76±0.01	1.44±0.02	1.69±0.03
Strain 12	0.99±0.02	0.20±0.07	0.24±0.00	0.78±0.01	0.39±0.01	0.32±0.00
Strain 13	1.11±0.07	0.15±0.00	0.23±0.01	1.02±0.17	0.68±0.06	0.35±0.03
Strain 14	1.42±0.00	0.13±0.00	0.13±0.00	0.72±0.01	1.74±0.02	1.89±0.03
Strain 15	1.69±0.08	0.13±0.00	0.14±0.00	1.22±0.19	2.04±0.10	1.89±0.08
Strain 16	0.92±0.03	0.16±0.02	0.30±0.00	0.99±0.43	0.61±0.05	0.71±0.06
Strain 17	1.78±0.05	0.13±0.01	0.25±0.00	1.78±0.05	1.26±0.16	0.49±0.05
Mean±SD	1.36±0.26	0.22±0.01	0.33±0.24	1.09±0.27	1.00±0.02	0.92±0.21
P values		.484	.951	.736	.657	.793

[a]Sprectrophotometric optical density of the strains in 600 nm

[b]Control pH: pH of the medium was used in the study.

SD. Standard Deviation. P values compared each bile concentrations with control, P=0.05.

Table 2. Results of the bile salt resistance of *Lactobacillus* isolated from Turkish local yogurt.

Strains	Control[b]	Bile concentration (%)[a]			
		0.15	0.20	0.30	0.40
Strain 1	1.55±0.00	1.18±0.00	1.08 ±0.04	1.16±0.01	0.86±0.04
Strain 2	1.59±0.01	1.31±0.07	0.75±0.05	0.85±0.04	0.39±0.01
Strain 3	1.48±0.02	1.08±0.00	1.04±0.00	1.14±0.01	0.73±0.02
Strain 4	1.54±0.06	1.24±0.07	1.09±0.01	1.12 ±0.02	0.55±0.03
Strain 5	1.33±0.05	1.39±0.14	0.35±0.03	0.48±0.03	0.35±0.00
Strain 6	1.64±0.00	1.21±0.01	0.73±0.00	0.51±0.02	0.37±0.00
Strain 7	1.64±0.06	1.28±0.02	0.45±0.02	0.46±0.03	0.35±0.06
Strain 8	1.62±0.02	1.28±0.08	0.95±0.02	1.07±0.01	0.64±0.03
Strain 9	1.94±0.06	0.33±0.01	0.31±0.00	0.32±0.00	0.34±0.00
Strain 10	1.44±0.03	1.15±0.03	1.07±0.01	0.90±0.03	0.90±0.02
Strain 11	1.52±0.02	1.18±0.00	1.04±0.00	1.12±0.01	0.68±0.02
Strain 12	1.44±0.06	1.18±0.02	0.42±0.03	0.39±0.01	0.34±0.00
Strain 13	1.45±0.04	1.08±0.03	1.10±0.02	1.06±0.04	0.86±0.00
Strain 14	1.70±0.01	1.67±0.00	1.60±0.00	1.59±0.00	1.50±0.01
Strain 15	1.52±0.07	1.01±0.04	0.68±0.02	0.47±0.02	0.25±0.05
Strain 16	1.60±0.02	1.38±0.07	1.09±0.05	1.21±0.00	0.73±0.03
Strain 17	1.42±0.06	0.91±0.03	0.58±0.02	0.30±0.00	0.28±0.00
Mean±SD	1.55±0.09	1.16±0.19	0.84±0.35	0.83±0.60	0.59±0.41
P values		.022	.506	.436	.467

[a]Concentration of the bile salt in percentage of the broth

[b]Control: As a control MRS broth not include bile salt was used in the study.

SD. Standard Deviation. P values compared each bile concentrations with control. P= 0.05.

G. A. Avci et al./ Hittite J Sci Eng, 2015, 2 (2) 1–185

strains from infant feces were tested for their ability to tolerance low pH (pH 3.0) and bile salt. Stay alive ratio of the test strains ranged between 0.01%-68.3% at pH 3.0 and 10.3%-57.4% at 0.15% bile salts [14]. It was also shown that *Lactococcus lactis* subsp. *lactis* lost viability at pH 2.0 and 0.2 % bile salts [15]. In another research, LAB strains isolated from local Korean food showed bile salt and acid resistance [16]. When we compare the results of the current work with those results in the literature, our strains have shown a good performance at low pH and bile salts.

Determination of EPS levels

Exopolysaccharides (EPS) are sugar polymers that secreted by the microorganism out of the cell. For microorganism, EPS provide resistance to harmful effects of nutrients and enzymatic activities in habitats and also EPS are important for cells in microbial aggregates to communicate each other [17]. In our study, EPS production capacities were determined spectrophotometrically using the phenol- sulphuric acid method and calculated. EPS production amounts of all *Lactobacillus* strains isolated from Turkish local yogurt was showed at Table 3. As shown Table 3, the lowest and highest EPS amounts were calculated respectively as 20.82 and 121.01 mg/L. In this study EPS production was observed in all strains but depending on the strain amount of EPS products were determined different. According to Korakli et al (2002), *Bifidobacterium* and *Lactobacillus* strains isolated from cereal products have EPS production activity, and this ensures fermentation and is important for typical flavor [3]. Vinderela G et al (2006), suggested that exopolysaccharide is protective immunity, maintaining intestinal homeostasis, enhancing the IgA production at both the small and large intestine level and influencing the systemic immunity through the cytokines released to the circulating blood [4]. Alp and Aslım (2010) reported that lactic acid bacteria and Bifidobacterium isolated from breast milk and infant feces showed acid resistance and bile salt tolerance. There has been a correlate with EPS production capacity and acid production-bile salt reduction activity [10].

CONCLUSION

Scientists have studied many times to determine the probiotic features of some *Lactobacillus* strains of LAB from the natural isolates to improve human health quality. These microorganisms are used biotechnological and industrial area and still we have not enough information their physiological features. These experiments provide an improvement to our knowledge about lactic acid bacteria as a probiotic microorganism. In this research, we determined to acid resistance, bile tolerance and EPS production capacity of *Lactobacillus* strains isolated from Turkish local yogurt. In Turkish population consuming yogurt and other local main Corum/Turkey. So, it is valuable in terms of the being an indicator of public health. Choosing most efficient strain from local yogurt and to develop its features may contribute to

Table 3. Exopolisaccaride (EPS) production amounts of *Lactobacillus* strains isolated from Turkish local yogurt.

Strains	OD_{490} Value	EPS Amount (mg/L)
Strain 1	0.278	34.15
Strain 2	0.258	30.24
Strain 3	0.247	28.07
Strain 4	0.250	28.67
Strain 5	0.317	41.02
Strain 6	0.210	20.82
Strain 7	0.226	23.96
Strain 8	0.395	57.09
Strain 9	0.317	41.8
Strain 10	0.383	54.74
Strain 11	0.367	51.60
Strain 12	0.374	52.98
Strain 13	0.389	55.92
Strain 14	0.328	43.96
Strain 15	0.366	51.41
Strain 16	0.721	121.01
Strain 17	0.404	58.86
Mean±SD	0.34±0.09	46.84±17.47

OD_{490}. Optical density was measured spectrophotometrically at 490 nanometer wavelength.
SD. Standard Deviation.

obtain healthier properties.

ACKNOWLEDGEMENTS

The authors wish to acknowledge this research experiment was a part of Hitit University Scientific Research Project Program (Project no.SYO01.13.001).

REFERENCES

1. Doleyres Y, Fliss I, Lacroix C. Increased stress tolerance of Bifidobacterium longum and Lactococcus lactis produced during continuous mixed−strain immobilized−cell fermentation. J Appl Microbiol 97 (2004) 527-39.
2. Sömer VF, Akpınar D, Başyiğit Kılıç G. *Lactobacillus casei*'nin Sağlık Üzerine Etkileri ve Gıda Endüstrisinde Kullanımı, Gıda 37 (3) (2012) 165−172.
3. Korakli M, Ganzle MG, Vogel RF. Metabolism by bifidobacteria and lactic acid bacteria of polysaccharides from wheat and rye, and exopolysaccharides produced by Lactobacillus sanfranciscensis, Journal of Applied Microbiology 92 (2002) 958-965.
4. Vinderola G, Perdigo´n G, Duarte J, Farnworth E, Matar C. Effects of the oral administration of the exopolysaccharide produced by Lactobacillus kefiranofaciens on the gut mucosal immunity. Cytokine 36 (2006) 254-260.
5. Sutherland IW. The biofilm matrix an immobilized but dynamic microbial environment. Trends in Microbiology. 9 (5) (2001) 222−227.
6. Welman AD, Maddox IS. Exopolysaccharides from lactic acid bacteria: perspectives and challenges. Trends in Biotechnology 21(6) (2003) 269−275.
7. Kranenburg van R, Vos HR, van Swam, Kleerebezem M, de Vos WM. Functional analysis of glycosyltransferases genes from Lactococcus lactis and other gram−positive cocci: complementation, expression, and diversity. J. Bacteriol. 181 (1999) 6347-6353.
8. Laws A, Gu Y, Marshall V. Biosynthesis, characterisation, and design of bacterial exopolysaccharides from lactic acid bacteria. Biotechnology Advances. 19 (2001) 597−625
9. Pan X, Chen F, Wu T, Tang H, Zhao Z. The acid, bile tolerance and antimicrobial property of Lactobacillus acidophilus NIT. Food Control 20 (2009) 598-602.
10. Alp G, Aslim B. Relationship between the resistance to bile salts and low pH with exopolysaccharide (EPS) production of Bifidobacterium spp. isolated from infants feces and breast milk, Anaerobe 16 (2010) 101-105.
11. Lin W, Hwang C, Chen L, Tsen H. Viable counts, characteristic evaluation for commercial lactic acid bacteria products. Food Microbiology 23(2006) 74-81.
12. Brink M, Todorov SD, Martin JH, Senekal M, Dicks LMT. The effect of probiotics on production of antimicrobial compounds, resistance to growth at low pH and in the intestinal mucus. J. Appl. Microbiol (2006) 1364−5072.
13. Noriega L, Gueimonde M, Sánchez B, Margolles A, de los Reyes−Gavilán CG. Effect of the adaptation to high bile salts concentrations on glycosidic activity, survival at low pH and cross−resistance to bile salts in *Bifidobacterium*. International Journal of Food Microbiology, 94 (1) (2004) 79-86.
14. Xanthopoulos V, Litopoulou−Tzanetaki E, Tzanetakis N. Characterization of *Lactobacillus* isolates from infant faeces as dietary adjuncts. Food Microbiol 17(2000) 205−215.
15. Kim W, Ren J, and W. Dunn (1999). Differentiation of *Lactococcus lactis* subspecies lactis and subspecies cremoris strains by their adaptive response to stress. Fems Microbiol. Lett. 171(1999) 57−6.
16. Chang JH, Shim YY, Cha SK, Chee1 KM. Probiotic characteristics of lactic acid bacteria isolated from kimchi. Journal of Applied Microbiology 109 (2010) 220-230.
17. Laspidou CS, Rittmann BE. A unified theory for extracellular polymeric substances, soluble microbial products, and active and inert biomass. Water Research. 36 (2002) 2711-2720.