Cihan University-Erbil Scientific Journal (CUESJ)

Research Article

Characterization of *Lactobacillus* Isolates from Human Mouth and Feces as Probiotics

Wala'a Sh. Ali*, Aya T. Abdulameer Reza

Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq

ABSTRACT

Probiotics are live microbes that give many health benefits to human beings and animals, the most studied and commonly used probiotics are Gram-positive bacteria; lactobacilli and bifidobacteria. At nowadays, *Lactobacillus* spp. constitute more than two-thirds of the total numbers of probiotic species. The present study aimed to characterize *Lactobacillus* that locally isolated from human mouth and feces as probiotics. A total of three *Lactobacillus* isolates; *Lactobacillus fermentum* Lb2, *Lactobacillus rhamnosus* Lb9, and *Lactobacillus paracasei* Lb10 were investigated in respect to acid and bile salts tolerance, antibiotics susceptibility, and cell surface hydrophobicity *in vitro* using bacterial adhesion to hydrocarbons method. In comparison with the other two isolates, the isolate *L. fermentum* Lb2 was able to grow in all pH values and in the presence of different concentrations of bile salts. Antibiotics susceptibility profile showed that the tested *Lactobacillus* isolates were sensitive to ampicillin, amoxicillin, and erythromycin, while they were resistant to the other antibiotics that used in this study. *L. fermentum* Lb2 exhibited high surface hydrophobicity (77.26%), while the other tested isolates; *L. rhamnosus* Lb9 and *L. paracasei* Lb10 revealed moderate adhesion abilities, 68.56% and 65%, respectively. *L. fermentum* Lb2 exhibited good probiotic behavior with respect to acid and bile salt tolerance as well as adhesion ability to hydrocarbons.

Keywords: Acid and bile salt tolerance, cell surface hydrophobicity, *Lactobacillus*, probiotic

INTRODUCTION

The attention of probiotic as an effective approach to prevent and treat a wide range of diseases is observed in recent years.[1] The term probiotic consists of two words which are pro, means for, and biotic, means life and totally means for life.[2] In 2001, the Food and Agriculture Organization of the United Nations and World Health Organization defined probiotics as "Live microorganisms which, when administered in adequate amounts, confer a health benefit on the host."[3] The US Food and Drug Administration stated that the probiotic is considered as generally recognized as safe.[4] Many studies showed that probiotics are used effectively to do many beneficial functions to the host[5-9] when they are taken in adequate quantity, and the recommended dosage of them is ranged usually between 10^8 and 10^{10} CFU/day.[10]

Probiotics can be found in a wide assortment of commercial dairy products[11] as well as they can be prepared as capsules, powder, granules, and fermented or pelleted feed.[12] Microorganisms are considered as probiotic when they have several criteria such as the ability to adhesion to gastrointestinal tract, the ability to tolerance acidity and bile salts, and the resistance to antibiotics[13] have health benefits for the host[14] and safety.[15] The most commonly used probiotics are *Lactobacillus* and *Bifidobacterium* species.[16] Lactobacilli are the largest group included in lactic acid bacteria[15] and have many functions, such as improvement of growth performance and health of gastrointestinal tract, this answers why they are among the bacteria mostly used as probiotics in animal feeds and human foods.[17,18] The aim of this study was to characterize three locally *Lactobacillus* isolates as probiotics.

MATERIALS AND METHODS

Bacterial Isolates

A total of three locally *Lactobacillus* isolates were used in this study; *Lactobacillus fermentum* Lb2 that isolated from the mouth, *Lactobacillus rhamnosus* Lb9 and *Lactobacillus paracasei* Lb10 that isolated from feces.[19]

Corresponding Author:
Dr. Wala'a Sh. Ali, Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.
E-mail: dr.walaashali@scbaghdad.edu.iq

Received: Apr 13, 2019
Accepted: Apr 19, 2019
Published: Jan 20, 2020

DOI: 10.24086/cuesj.v4n1y2020.pp7-12

Acid Tolerance Test

Fresh broth cultures of *Lactobacillus* isolates (Lb2, Lb9, and Lb10) were used at concentration of 1.5×10^8 CFU/ml according to McFarland tube No. 0.5 (Hardy Diagnostics, Santa Maria). Serial dilutions were made in tubes contain MRS broth (Oxoid, Germany), then bacteria were transferred individually to MRS broth at various pH values (2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, and 6), and tubes were incubated at different times (1, 1.5, 2, 2.5, and 3 h) h; thereafter, 0.1 ml from each culture was taken and spread on MRS agar (Oxoid, Germany) with two replicates. Plates were incubated anaerobically for 24 h at 35°C. The pronounced colonies were counted, and results were recorded corresponding to control.[20]

Bile Salt Tolerance Test

Fresh broth cultures of *Lactobacillus* isolates (Lb2, Lb9, and Lb10) were used at the concentration of 1.5×10^8 CFU/ml according to McFarland tube No. 0.5. Serial dilutions were made in tubes contain MRS broth, then bacteria were transferred individually to MRS broth supplemented with various concentrations of bile salts (Difco, USA) (0.3%, 0.5%, and 0.7%), tubes were incubated at different times (1, 1.5, 2, 2.5, and 3 h); thereafter, 0.1 ml from each culture was taken and spread on MRS agar with two replicates, and then plates were incubated anaerobically for 24 h at 35°C. The pronounced colonies were counted, and results were recorded corresponding to control.[21] The optical density of bacterial isolates growth in each bile salt concentration was measured at wavelength 660 nm after 3 h of incubation.

Antibiotics Susceptibility Test

Fresh broth cultures of *Lactobacillus* isolates (Lb2, Lb9, and Lb10) were used at concentration of 1.5×10^8 CFU/ml according to McFarland tube No 0.5; thereafter, 0.1 ml from each culture was taken and spread on MRS agar, and then antibiotics discs (25 μg ampicillin, 25 μg amoxicillin, 10 μg tetracycline, 1.25/23.75 μg trimethoprim-sulfamethoxazole, 10 μg erythromycin, 10 μg gentamycin, 25 μg streptomycin, 10 μg amikacin, 10 μg neomycin, and 10 μg chloramphenicol) (Bioanalyse, Turkey) were gently placed on MRS agar plates, plates were incubated anaerobically at 35°C for 24 h. The inhibition zones were measured and recorded; the work was done with two replicates.[22]

Cell Surface Hydrophobicity Test

It was determined *in vitro* using bacterial adhesion to hydrocarbons (BATH) method,[23] a fresh cultures of *Lactobacillus* isolates were subjected to centrifugation (6000 rpm, 10 min), cells were washed by phosphate buffer saline (Oxoid, Germany), resuspended in the same buffer and the optical densities of suspensions were adjusted to 0.8–1 at (OD 560), and the data were recorded as A 0.6 ml of *Lactobacillus* suspensions were mixed with 1.2 ml of n-hexadecane (Sigma, USA), gently vortexed, and tubes were incubated anaerobically at 35°C for 30min. The mixtures were vortexed rigorously for 2 min and then allowed to stand for 15 min at room temperature to ensure complete separation of the organic and the aqueous phase. The absorbance of aqueous phase was measured at 560 nm and recorded as A, the affinity to solvent was expressed using the formula:

$$\text{Hydrophobicity percentage} = \frac{(\text{A0-A})}{\text{A0}} \times 100$$

A0, before adding n-hexadecane; A, after adding n-hexadecane.

Statistical Analysis

Statistical Analysis System[24] program was used to show the effect of different factors in study parameters. Furthermore, least significant difference (LSD) test was used to significant compare between means in this study.

RESULTS AND DISCUSSION

Probiotic strains must be able to survive and colonize in the presence of acidic conditions and bile salt so as to travel through the gastrointestinal tract and reach to digestive tract where they give their therapeutic effect to host.[25] In current study, three *Lactobacillus* isolates were subjected to different pH values with different incubation periods to examine their ability to tolerate acidic conditions. According to the results in Table 1, all three isolates were unable to grow at pH 2, while they were able to grow at all times of incubation at pH 4, 4.5, and 5 with a decline in their growth at pH 6 after its rise at pH 5.5. At pH 5, there was no significant difference in the growth of the three isolates except the 1st h of incubation with significant difference in time 3 h for Lb9.

The gradual decrease in the survival rate of bacterial growth, from pH 5 down to pH 2, as well as the decrease in bacterial growth at pH 6 after its increasing at pH 5.5 can be observed, it seemed there is an affinity of the three isolates to pH 5.5 where their survival percentage reached the highest at this pH value, these results are similar to previous studies that used pH 5.5 in preparation of media as it allows optimum growth for *Lactobacillus*.[26,27] At pH 6, the reduction of the growth of the three isolates is noticeable at the 1st h of incubation while there was no significant difference in their growth in times 1.5 and 2 h for Lb2 and in times 2 and 2.5 h for Lb10. The significant decrease in the bacterial growth at the 1st h of incubation is due to the sudden change in pH as reported by Fernández-Calviño and Bååth.[28]

The ability of three *Lactobacillus* isolates to survive at three different concentrations of bile salts for different incubation periods was examined. The results in Table 2 show that all isolates were able to survive at all bile salts concentrations. The results of this study are agreed with previous studies used 0.3% and 0.5% of bile salts concentrations for 2.5 h of incubation.[29,30] The gradual decrease in the survival rate of bacterial growth as a result of the increase of concentration of bile salts can be observed. The significant decrease in bacterial growth at the 1st h of incubation is due to the sudden exposure to bile salts stress while there was no significant decrease in bacterial growth after the 1st h of incubation particularly at high concentration of bile salts. The resistance of *Lactobacillus* to bile salts may be due to different mechanisms such as changes in the composition of cell membrane and cell wall, active efflux of bile acids, and bile salt hydrolysis.[31] Figure 1 shows

Table 1: Acid tolerance percentages of *Lactobacillus* isolates at different pH values

pH	h	Mean of viable count (log10 CFU/ml)			Survival percentage		
		Lb2	Lb9	Lb10	Lb2	Lb9	Lb10
2.5	1	8.505^{B}	8.612^{B}	-	2	3	0
3	1	8.748^{B}	8.959^{B}	8.643^{B}			
	1.5	-	8.913^{a}	-	4	25	4
	2	-	8.838^{a}	-			
	2.5	-	8.643^{b}	-			
3.5	1	8.851^{B}	9.017^{B}	8.799^{B}			
	1.5	8.740^{a}	8.949^{a}	8.505^{b}			
	2	-	8.886^{b}	-	10	33	9
	2.5	-	8.770^{b}	-			
	3	-	8.643^{b}	-			
4	1	9.120^{B}	9.086^{B}	9.004^{B}			
	1.5	9.110^{a}	9.079^{a}	8.959^{a}			
	2	9.008^{b}	9.004^{a}	8.869^{a}	46	45	39
	2.5	8.954^{b}	8.949^{b}	8.819^{b}			
	3	8.919^{b}	8.892^{b}	8.698^{b}			
4.5	1	9.178^{B}	9.264^{B}	9.093^{B}			
	1.5	9.152^{a}	9.136^{b}	9.096^{a}			
	2	9.123^{a}	9.113^{b}	9.079^{a}	55	59	61
	2.5	9.079^{b}	9.071^{b}	9.060^{a}			
	3	8.963^{b}	9.004^{b}	9.045^{a}			
5	1	9.274^{B}	9.204^{B}	9.152^{B}			
	1.5	9.276^{a}	9.198^{a}	9.130^{a}			
	2	9.247^{a}	9.146^{a}	9.113^{a}	77	64	66
	2.5	9.235^{a}	9.130^{a}	9.086^{a}			
	3	9.227^{a}	9.123^{b}	9.082^{a}			
5.5	1	9.298^{B}	9.301^{A}	9.250^{A}			
	1.5	9.294^{a}	9.278^{a}	9.230^{a}			
	2	9.271^{a}	9.247^{a}	9.204^{a}	81	80	80
	2.5	9.255^{a}	9.230^{b}	9.158^{b}			
	3	9.247^{a}	9.225^{b}	9.130^{b}			
6	1	9.235^{B}	9.206^{B}	9.117^{B}			
	1.5	9.220^{a}	9.146^{a}	9.082^{a}			
	2	9.178^{a}	9.127^{b}	9.056^{a}	65	59	58
	2.5	9.143^{b}	9.079^{b}	9.045^{a}			
	3	9.079^{b}	9.075^{b}	8.977^{b}			

Capital letters (A and B) indicate differences between the numbers of 1 h of incubation and control while the small letters indicate differences between the numbers of current hour and 1 h of incubation at $P<0.05$ based on the LSD test; ^{A,a}No significant difference; ^{B,b}Significant difference; -: No viable cells; Lb2: *L. fermentum*; Lb9: *L. rhamnosus*; Lb10: *L. paracasei*; missing pH (2) and hours, no viable cells

the tolerance of *Lactobacillus* to three bile salts concentrations after 3 h of incubation.

For probiotic bacteria to be active for a long time in the gastrointestinal tract, they should be able to resist administrated antibiotics. In this study, ten antibiotics were used. The results in Figure 2 show that Lb2, Lb9, and Lb10 were highly sensitive to ampicillin, amoxicillin, and erythromycin; this was in accordance with reported by other authors.[32-35] For Lb2, the inhibition zone of ampicillin was higher than that of amoxicillin and erythromycin while the inhibition zones of these three antibiotics were the same for Lb9 while for Lb10, amoxicillin made a higher inhibition zone than other two antibiotics. Lb9 was resistant to trimethoprim-sulfamethoxazole, amikacin, and neomycin where there was no inhibition zone; these results are similar to those of Coppola *et al*.[36]

Table 2: Bile salt tolerance percentages of *Lactobacillus* isolates at different bile salts concentrations

Bile salt concentrations %	h	Mean of viable count (log10 CFU/ml)			Survival percentage		
		Lb2	Lb9	Lb10	Lb2	Lb9	Lb10
0.3	1	9.385^{A}	9.372^{B}	9.245^{B}	70	68	58
	1.5	9.369^{a}	9.332^{a}	9.212^{a}			
	2	9.235^{b}	9.278^{b}	9.133^{b}			
	2.5	9.193^{b}	9.262^{b}	9.130^{b}			
	3	9.146^{b}	9.260^{b}	9.079^{b}			
0.5	1	9.305^{B}	9.324^{B}	9.103^{B}	52	49	43
	1.5	9.176^{b}	9.206^{b}	9.089^{a}			
	2	9.133^{b}	9.146^{b}	9.021^{a}			
	2.5	9.082^{b}	9.082^{b}	8.924^{b}			
	3	8.991^{b}	8.968^{b}	9.004^{a}			
0.7	1	9.170^{B}	9.060^{B}	9.068^{B}	42	30	44
	1.5	9.117^{a}	9.029^{a}	9.060^{a}			
	2	9.045^{b}	8.954^{a}	9.056^{a}			
	2.5	9.029^{b}	8.857^{b}	9.037^{a}			
	3	8.908^{b}	8.851^{b}	9.017^{a}			

Capital letters (A and B) indicate differences between the numbers of 1 h of incubation and control while the small letters indicate differences between the numbers of current hour and 1 h of incubation at $P<0.05$ based on the LSD test; ^{A,a}No significant difference; ^{B,b}Significant difference ($P<0.05$); Lb2: *L. fermentum*; Lb9: *L. rhamnosus*; Lb10: *L. paracasei*

Table 3: Cell surface hydrophobicity of *Lactobacillus* isolates

***Lactobacillus* isolates**	Absorbance		Hydrophobicity percentage
	A0	A	
Lactobacillus fermentum Lb2	0.8273	0.1881	77.26 (high)
Lactobacillus rhamnosus Lb9	0.9965	0.3132	68.56 (medium)
Lactobacillus paracasei Lb10	0.8206	0.2872	65.00 (medium)

A0: Before adding n-hexadecane; A: After adding n-hexadecane; low: 0–35%; medium: 36–70%; high: 71–100%

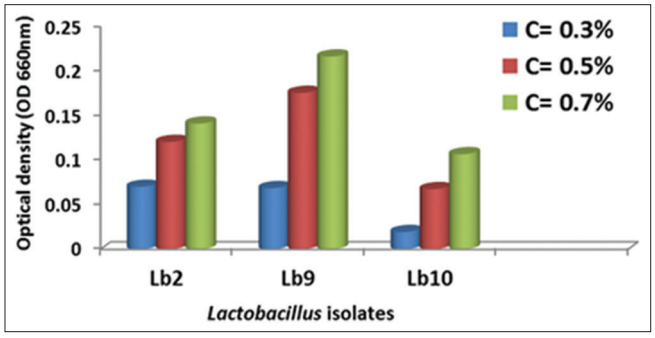

Figure 1: Tolerance of *Lactobacillus* isolates; Lb2, *Lactobacillus fermentum*; Lb9, *Lactobacillus rhamnosus;* and Lb10, *Lactobacillus paracasei* to bile salts concentrations (0.3%, 0.5%, and 0.7%)

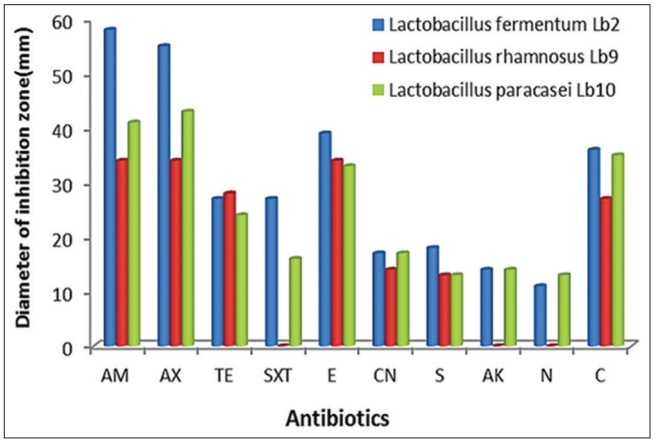

Figure 2: Antibiotics susceptibility test of *Lactobacillus* isolates; AM: Ampicillin; AX: Amoxicillin; TE: Tetracycline; SXT: Trimethoprim-sulfamethoxazole; E: Erythromycin; CN: Gentamycin; S: Streptomycin; AK: Amikacin; N: Neomycin; C: Chloramphenicol

Cell surface hydrophobicity of *Lactobacillus* isolates was determined to correlate the data with their ability to adhere to the intestinal epithelium. Cell surface hydrophobicity was determined using the method of BATH. The percent cell surface hydrophobicity was calculated, as shown in Table 3. According to three levels of hydrophobicity (low: 0–35%, medium: 36–70%, and high: 71–100%) that determined by Ocana *et al.*[37] Lb2 showed a high hydrophobicity which was 77.26% while Lb9 and Lb10 showed medium hydrophobicity with percentage of 68.56% and 65%, respectively, the results of Lb2

and Lb9 are similar to previous study of García *et al*.[35] The BATH test quantifies the surface hydrophobicity of bacteria, and it has widely been used to estimate the physicochemical component in adhesion ability of bacterial strains.[38] Hence, based on the hydrophobicity or the charge of the bacterial outer layer, the adhesion ability to hydrophobic surfaces such as mucus can be characterized indirectly.[39]

However, consumption products that contain probiotic bacteria such as dairy products and fermented vegetables as well as eat healthy foods rich in fibers and prebiotics will contribute to increase the numbers of these useful bacteria in our gastrointestinal tract, and this lead to reestablishment of normal microbiota balance and enhancement their activities.

ACKNOWLEDGMENT

We would like to thank Dr. Nasr Noori Al-Anbari for his help in statistical analysis.

CONCLUSION

Depending on the results of this study, the bacterial isolate *L. fermentum* Lb2 showed up good probiotic behavior in respect of acid and bile salt tolerance and adhesion ability to hydrocarbons. However, more studies are required about this isolate and thus could be good candidate to use as probiotic.

REFERENCES

1. W. S. Ali and N. N. Mahmood. "Probiotic therapy: Review". *Iraqi Journal of Medical Sciences*, vol. 14, no. 1, pp. 1-6, 2016.
2. S. Fijan. "Microorganisms with claimed probiotic properties: An overview of recent literature". *International Journal of Environmental Research and Public Health*, vol. 11, no. 5, pp. 4745-4767, 2014.
3. R. Frei, M. Akdisa and L. O'Mahony. "Prebiotics, probiotics, synbiotics, and the immune system: Experimental data and clinical evidence". *Current Opinion in Gastroenterology*, vol. 31, pp. 1-6, 2015.
4. S. Doron and D. R. Snydman. "Risk and safety of probiotics". *Clinical Infectious Diseases*, vol. 60 Suppl 2, pp. S129-S134, 2015.
5. S. Guandalini. "Probiotics for prevention and treatment of diarrhea". *Journal of Clinical Gastroenterology*, vol. 45, pp. S149-S153, 2011.
6. A. Patel, N. Shah and J. B. Prajapati. "Clinical application of probiotics in the treatment of *Helicobacter pylori* infection- a brief review". *Journal of Microbiology, Immunology and Infection*, vol. 47, no. 5, pp. 429-437, 2014.
7. J. M. Kim and Y. J. Park. "Probiotics in the prevention and treatment of postmenopausal vaginal infections: Review". *Journal of Menopausal Medicine*, vol. 23, no. 3, pp. 139-145, 2017.
8. S. J. Oak and R. Jha. "The effects of probiotics in lactose intolerance: A systematic review". *Critical Reviews in Food Science and Nutrition*, vol. 59, pp. 1-9, 2018.
9. B. S. Sivamaruthi. "A comprehensive review on clinical outcome of probiotic and synbiotic therapy for inflammatory bowel diseases". *Asian Pacific Journal of Tropical Biomedicine*, vol. 8, no. 3, pp. 179-186, 2018.
10. S. Sarkar. "Worldwide consumer's awareness and inclination towards healthful food products". *International Journal of Food Sciences and Nutrition*, vol. 5, no. 2e, p. 1, 2016.
11. G. Gardiner, C. Stanton, P. B. Lynch, J. K. Collins, G. Fitzgerald and R. P. Ross. "Evaluation of cheddar cheese as a food carrier for delivery of a probiotic strain to the gastrointestinal tract". *Journal of Dairy Science*, vol. 82, no. 7, pp. 1379-1387, 1999.
12. Y. Ohashi and K. Ushida. "Health-beneficial effects of probiotics: Its mode of action". *Animal Science Journal*, vol. 80, pp. 361-371, 2009.
13. N. Farahmand. "Characterization of Probiotic *Lactobacillus* spp. isolates from Commercial Fermented Milks", Ph.D thesis, London Metropolitan University, England, 2015.
14. C. Dunne, L. O'Mahony, L. Murphy, G. Thornton, D. Morrissey, S. O'Halloran, M. Feeney, S. Flynn, G. Fitzgerald, C. Daly, B. Kiely, G. C. O'Sullivan, F. Shanahan and J. K. Collins. "*In vitro* selection criteria for probiotic bacteria of human origin: Correlation with *in vivo* findings". *American Journal of Clinical Nutrition*, vol. 73, no. 2, pp. 386s-392s, 2001.
15. S. Gulel. "Molecular Identification and Properties of '*Lactobacillus acidophilus*' group Isolates from Turkish Kefir," M.Sc.thesis, Middle East Technical University, Turkey, 2014.
16. M. A. Ciorba. "A gastroenterologist's guide to probiotics". *Clinical Gastroenterology and Hepatology*, vol. 10, no. 9, pp. 960-968, 2012.
17. V. Coeuret, M. Gueguen and J. P. Vernoux. "Numbers and strains of lactobacilli in some probiotic products". *International Journal of Food Microbiology*, vol. 97, no. 2, pp. 147-156, 2004.
18. R. Dowarah, A. K. Verma and N. Agarwal. "The use of *Lactobacillus* as an alternative of antibiotic growth promoters in pigs: A review". *Animal Nutrition*, vol. 3, no. 1, pp. 1-6, 2017.
19. A. T. A. Reza and W. S. Ali. "Antibacterial activity of *Lactobacillus* spp. against *Listeria monocytogenes*". *Indian Journal of Public Health Research and Development*, In Press.
20. W. P. Charteris, P. M. Kelly, L. Morelli and J. K. Collins. "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*, vol. 84, no. 5, pp. 759-768, 1998.
21. M. F. Fernandez, S. Boris and C. Barbes. "Probiotic properties of human lactobacilli strains to be used in the gastrointestinal tract". *Journal of Applied Microbiology*, vol. 94, no. 3, pp. 449-455, 2003.
22. W. P. Charteris, P. M. Kelly, L. Morelli and J. K. Collins. "Antibiotic susceptibility of potentially probiotic *Lactobacillus* species". *Journal of Food Protection*, vol. 61, no. 12, pp. 1636-1643, 1998.
23. M. Rosenberg, D. Gutnick and E. Rosenberg. "Adherence of bacteria to hydrocarbons: A simple method for measuring cell-surface hydrophobicity". *FEMS Microbiology Letters*, vol. 9, no. 1, pp. 29-33, 1980.
24. SAS. Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. Cary. N.C. USA: SAS. Institute Inc., 2012.
25. E. Tuomola, R. Crittenden, M. Playne, E. Isolauri and S. Salminen. "Quality assurance criteria for probiotic bacteria". *American Journal of Clinical Nutrition*, vol. 73, no. 2, pp. 393s-398s, 2001.
26. L. Samaranayake. "*Essential Microbiology for Dentistry E-Book*", 4th ed. Amsterdam, Netherlands: Elsevier Health Sciences, 2011.
27. A. M. Grumezescu and A. M. Holban. "*Advances in Biotechnology for Food Industry*". Cambridge, USA: Academic Press, 2018.
28. D. Fernández-Calviño and E. Bååth. "Growth response of the bacterial community to pH in soils differing in pH". *FEMS Microbiology Ecology*, vol. 73, pp. 149-156, 2010.
29. M. Mirlohi, S. Soleimanian-Zad, S. Dokhani, M. Sheikh-Zeinodin and A. Abghary. "Investigation of acid and bile tolerance of native lactobacilli isolated from fecal samples and commercial probiotics by growth and survival studies". *Iranian Journal of Biotechnology*, vol. 7, no. 4, pp. 233-240, 2009.
30. M. Tavakoli1, Z. Hamidi-Esfahani, M. A. Hejazi, M. H. Azizi and S. Abbasi. "Characterization of probiotic abilities of lactobacilli isolated from iranian koozeh traditional cheese". *Polish Journal of Food and Nutrition Sciences*, vol. 67, no. 1, pp. 41-48, 2017.
31. L. Ruiz, A. Margolles and B. Sánchez. "Bile resistance mechanisms

in *Lactobacillus* and *Bifidobacterium*". *Frontiers in Microbiology*, vol. 4, pp. 1-8, 2013.

32. N. Zdolec, I. Filipović, Ž. C. Fleck, A. Marić, D. Jankuloski, L. Kozačinski and B. Njari. "Antimicrobial susceptibility of lactic acid bacteria isolated from fermented sausages and raw cheese". *Veterinarski Arhiv*, vol. 81, no. 1, pp. 133-141, 2011.
33. S. K. Allameh, F. M. Yusoff, H. M. Daud, E. Ringø, A. Ideris and C. R. Saad. "Characterization of a probiotic *Lactobacillus fermentum* isolated from snakehead, channa striatus, stomach". *Journal of the World Aquaculture Society*, vol. 44, no. 6, pp. 835-844, 2013.
34. R. Georgieva, L. Yocheva, L. Tserovska, G. Zhelezova, N. Stefanova, A. Atanasova, A. Danguleva, G. Ivanova, N. Karapetkov, N. Rumyan and E. Karaivanova. "Antimicrobial activity and antibiotic susceptibility of *Lactobacillus* and *Bifidobacterium* spp. intended for use as starter and probiotic cultures". *Biotechnology and Biotechnological Equipment*, vol. 29, no. 1, pp. 84-91, 2015.
35. A. García, K. Navarro, E. Sanhueza, S. Pineda, E. Pastene, M. Quezada, K. Henríquez, A. Karlyshev, J. Villena and C. González. "Characterization of *Lactobacillus fermentum* UCO-979C, a probiotic strain with a potent anti-*Helicobacter pylori* activity". *Electronic Journal of Biotechnology*, vol. 25, pp. 75-83, 2017.
36. R. Coppola, M. Succi, P. Tremonte, A. Reale, G. Salzano and E. Sorrentino. "Antibiotic susceptibility of *Lactobacillus rhamnosus* strains isolated from parmigiano reggiano cheese". *Lait*, vol. 85, no. 3, pp. 193-204, 2005.
37. V. S. Ocana, E. Bru, A. A. De Ruiz Holgado and M. E. Nader-Macias. "Surface characteristics of lactobacilli isolated from human vagina". *Journal of General and Applied Microbiology*, vol. 45, no. 5, pp. 203-212, 1999.
38. T. De Wouters, C. Jans, T. Niederberger, P. Fischer and P. A. Ruhs. "Adhesion potential of intestinal microbes predicted by physico-chemical characterization methods". *PLoS One*, vol. 10, no. 8, pp. 1-17, 2015.
39. M. C. Van Loosdrecht, J. Lyklema, W. Norde, G. Schraa and A. J. Zehnder. "The role of bacterial cell wall hydrophobicity in adhesion". *Applied and Environmental Microbiology*, vol. 53, no. 8, pp. 1893-1897, 1987.