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In vitro evaluation of probiotic properties of commercial strains *Lactobacillus plantarum* and *Bifidobacterium animalis*subsp. *Lactis*

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ABSTRACT

Probiotics are recognized as live microorganisms that confer a health benefit to the host when administered in adequate amounts. The present study aimed to evaluate *in vitro* probiotic properties of two commercial probiotic strains, *Bifidobacterium animalis*subsp. *lactis* BB-12 and *Lactobacillus plantarum* ATCC 14917. Our results indicated that the selected strains showed high resistance to acid, bile salts,and lysozyme. In general, they showed good adaptation to simulated gastric and intestinal juices (more than 85% could survive) which guarantees their survivalin the gastrointestinal tract. Moreover, *Bifidobacterium animalis*subsp. *lactis* BB-12 showed the highest hydrophobicity (59.75%) and auto-aggregation (51.42%) but the lowest adhesion to the human intestinal HT-29 cell line (8.35%). Furthermore, they both had β-galactosidase activity and were resistant to penicillin, vancomycin, and tetracycline. Our results indicated that *Lactobacillus plantarum* ATCC 14917 had better characteristics of a probiotic compared to *Bifidobacterium animalis*subsp. *lactis* BB-12.

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1. Introduction

In recent years, the world's attention has increased to the use of practical foods containing probiotic bacteria to improve health and prevent diseases. According to the definition of the World Health Organization[1] (WHO) and Food and Agriculture Organization[2](FAO) in 2001, probiotics are live microorganisms that, when consumed in sufficient amounts ($CFU.gr^{-1}10^7$), will have beneficial effects on the health of the host body. Bacteria with probiotic properties often belong to*Lactic acid bacteria*And*Bifidobacteria* and are widely used in dairy products and probiotic drinks, as well as capsules, powders and food supplements. The benefits of probiotics can be reduced symptoms of lactose intolerance, reducing the period of acute gastroenteritis infection in children, improving the condition of the digestive system (traveler's diarrhea and Diarrhea caused by antibiotics), reducing the prevalence of vaginal infections, increasing immune function and reducing cholesterol and fat levels. Daily consumption of at least 10 CFU^7-10^9 per day is necessary for adequate colonization of probiotics in the intestine [1].

Common bacterial strains used as probiotics often belong to*Lactobacilli* And*Bifidobacteria* are.*Lactobacilli* to branch*Firmicute*And*Bifidobacteria* to branch*Actinobacter*belongs to. Both are Gram-positive microorganisms, with the difference that the molar percentage of C+G in Bifidobacteria (more than 60%) is higher than that of Lactobacillus (33-39%) and the catabolic pathway of Bifidobacteria is carried out through Bifidium hexose shunt and fructose-6-phosphoctolase enzyme [2].*Bifidobacterium animalis* subspecies*Lactis*[3] Between*Bifidobacteria* It has the smallest genome size at 193,269 bp and has several subspecies such as AD011, BL-04, DSM 10140, V9, HN019 and BB-12.*Bifidobacterium animalis subspecies lactis*BB-12 was first isolated and identified from the feces of infants in 1899. This strain was deposited in the Christian Hansen Cell Culture Bank in 1983. Clinical studies have shown that this strain is able to survive in the digestive tract and produce beneficial effects [3].*Bacillus plantarum lactiplantarum*[4] (which before*Lactobacillus plantarum*was called) is a non-fermenting bacterium with high adaptability that can be isolated from different habitats such as milk, fruit, cereals, bee pollen and fresh meat. It is also found as a common host in the digestive system of humans and other animals [4]. Among lactobacilli, it has the largest genome with 325,387 base pairs and produces a complete set of enzymes and proteins. Lactobacillus splantaromenis has good resistance against similar conditions of the digestive system (stomach acid and bile acids) [5].

The first necessity for the development of a probiotic food product is the selection of the right probiotic strain. Considering that the occurrence of probiotic properties is completely dependent on the gender and strain; The purpose of the current research is to evaluate the probiotic characteristics of two commercial strains*Bifidobacterium animalis* subspecies*Lactis*BB-12 в*Lactobacillus splantarum*ATCC14917 and comparing the functional characteristics of these two commercial strains in vitro. The survival rate in similar conditions of the digestive system and tolerance of acid and bile are among the main criteria for assessing the potential of probiotics, which were investigated in this research. Also, resistance to antibiotics, resistance to lysozyme, hydrophobicity, self-aggregation, beta-galactosidase activity and the ability of the strains to bind to intestinal epithelial cells have also been studied in this research.

2- Materials and methods

2-1- Activation of bacteria

Lyophilized package*Bifidobacterium animalis* under cheek*Lactis*12 BB- From Peshgaman Shadiq Company (representative of Christian Hansen Danish company in Iran) and*Lactobacillus plantarum*ATCC 14917 was obtained from the microbial collection of Ferdowsi University of Mashhad. In order to activate, bacterial stock*Lactobacillus splantarum*ATCC 14917 after leaving the frozen state on MRS culture medium[5] (MRS) agar (Iberesco, Iran) was surface cultured and incubated at 37°C for 48 hours in an

[1].World health organization

[2]. Food and agriculture organization

[3]. *Bifidobacterium animalis subsp. of milk*

[4]. *Lactiplantibacillus plants*

[5]. de Man, Rogosa and Sharpe (MRS)

atmosphere containing 10% carbon dioxide. Lyophilized package of bacteria*Bifidobacterium animalis* subspecies*Lactis*BB-12 Also, according to the instructions for activation after leaving the frozen state in the liquid MRS culture medium containing L-cysteine hydrochloride (0.05%, Sigma-Aldrich, USA) (MRSC) under completely anaerobic conditions in the presence of Gaspak A (Merck, Germany) at a temperature 37°C for 48 hours.

2-2- Preparation of microbial suspension

To prepare microbial suspension,*Lactobacillus plantarum*ATCC14917 in MRS broth and*Bifidobacterium animalis* subspecies*Lactis*BB-12 were incubated aerobically and anaerobically in the MRSC broth environment at 37°C, respectively, at the right time. Then the grown culture medium was centrifuged for 10 minutes (4650.88×g). After the supernatant was drained and washed twice with phosphate buffer, the cell sediment was redissolved in this solution. This suspension was used in all tests.

2-3- Evaluation of probiotic potential

2-3-1- Determination of resistance to acid

First, the culture medium of MRS The liquid was adjusted to pH 2 and 3 using hydrochloric acid (3 M) and sodium hydroxide (1 M). The cultures were autoclaved at 121°C for 15 minutes. After preparing the microbial suspension, a concentration equivalent to half McFarland was inoculated to two percent (v/v) of the culture medium. The survival rate of the strains after 60 minutes (pH=2) and 180 minutes (pH=3) of warm housing at 37°C under optimal conditions of each of the strains through serial dilution and point culture on MRS and MRSC solid was calculated (relation 1) [6].

(Relation 1) $100\times(N/N_0)$ = percentage of survival

where N_0 and N It is the number of primary and secondary colonies, respectively.

2-3-2- Determination of resistance to bile salt

The ability of strains to grow after inoculation of 2% (v/v) of strains cultured for 16-18 hours in MRS medium وMRSC The liquid was examined in the presence of 0.3, 0.5 and 1% (weight/volume) of bovine bile salt (Sigma, USA). After 24 hours of incubation at 37°C, the resistance of strains with serial dilution and point culture on MRS culture medium and MRSC Agar was evaluated. Culture medium without bile was also used as a control medium for comparison [6].

2-3-3- Determination of resistance in simulated conditions of the digestive system

Simulated gastric and intestinal juice in vitro from pepsin solubilization ($^{1-}$mg.ml3; Sigma) and Pancreatin ($^{1-}$1 mg.ml, Sigma) was prepared in sodium chloride salt solution (0.5% w/v) and sterilized with a sterile syringe filter. Next, pH Simulated gastric and intestinal juice using hydrochloric acid (3 M) and sodium hydroxide (1 M) respectively at Ph=3 =۸ وpH were adjusted Then by inoculating 200 microliters of suspension prepared from bacterial strains obtained from 16-18 hours of culture in phosphate buffered saline solution in 1 ml of simulated stomach and intestinal juice and adding 300 microliters of sodium chloride saline solution (0.5% (weight/volume))) were well mixed and kept in a hot house at 37°C for 180 minutes (through the stomach) and 240 minutes (through the small intestine). Resistance of strains after hothouse with serial dilution and spot culture on MRS and MRSC culture media Agar was examined. The number of live cells (cfu/ml) in the suspension at zero time was counted on the agar culture medium [6, 7].

2-3-4- Determination of resistance to lysozyme

To perform this test, the strains were cultured for 16-18 hours after centrifugation and separation of the cell mass and washing them in saline phosphate buffer (pH: 7.4±0.1), dissolved in Ringer's solution. Then, 10 microliters of bacterial suspension in a sterile electrolyte solution (CaCl2: 0.22 g/l, NaCl: 6.2 g/l, KCl: 2.2 g/l, NaHCO3: 1.2 g/l) containing 100 mg/liter lysozyme was inoculated for one hour. It was kept in a greenhouse at a temperature of 37°C. Electrolytic solution containing suspension without lysozyme was selected as a control sample. Counting of live cells by Kant plate method on MRS/MRSC Agar was done and the survival percentage of the strains after one hour was calculated in terms of log CFU/ml compared to zero time [8].

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2-3-5-Evaluation of the property of hydrophobicity

According to the method of Winderola and Renheimer (2003) after centrifugation and isolation of cells obtained from 16-18 hours culture of strains in MRS medium and MRSC The liquid was heated at 37°C and washed twice with potassium dihydrogen phosphate buffer, cell suspension was prepared in the same buffer. The absorbance of the cell suspension at 560 nm is set to approximately one. 3 ml of cell suspension was mixed with 0.6 ml of n-hexadecane (polar solvent) for 120 seconds. With the passage of time at 37°C, two phases were separated and the supernatant was carefully collected. The decrease in absorption in the underlying liquid was calculated as the hydrophobicity of the cell surface (Relation 2) [9].

(Relation 2) $[(A_0-A)/A_0] \times 100 =$ hydrophobicity percentage

that A_0 and A is absorption before and after extraction with n-hexadecane, respectively.

2-3-6-The test of its mass

The strains with the highest percentage of self-accumulation are considered good probiotic strains, therefore, to evaluate this characteristic, the suspension of grown cells obtained from 16-18-hour culture in phosphate buffered saline solution was prepared to obtain an optical density of 0.25 at a wavelength of 600 nm. The bacterial suspension (4 ml) was mixed for 10 seconds and kept in a greenhouse at 30°C. The absorbance of the samples was measured at 600 nm at the beginning, after 3 and 24 hours, and the percentage of spontaneous aggregation was calculated using equation 3 [10]:

(Relation 3) $[(A_0-A)/A_0] \times 100$ = percentage of its mass

that A_0 and A is the absorption at the beginning and a certain time respectively.

2-3-7- Determining resistance to antibiotics

In order to evaluate the sensitivity of the strains to antibiotics from the antibiogram test based on the disc diffusion resistance pattern[6] Kirby-Bauer method was used. For this purpose, a suspension with 0.5 McFarland turbidity was prepared from the 18-hour cultivation of bacterial strains. After inoculation of each of the strains in the amount of 100 microliters in Mueller Hinton Agar medium[7] (with and without L-cysteine hydrochloride at the rate of 0.05% (volume/volume)) and placing antibiotic discs with the help of sterile forceps on the surface of the culture medium and stabilizing it (15 minutes at room temperature), the plates at C° 37 were kept in a greenhouse for 48 hours under optimal growth conditions of each of the strains. In this test, 7 types of different antibiotic discs including (micrograms per disc): gentamicin[8] (10), erythromycin[9] (15), amoxicillin[10] (25), tetracycline[11] (10), Coloramphenical[12] (30), vancomycin[13] (30) and penicillin[14] (10) was used. Observing a non-growth halo of more than 6 mm was considered as a strong antagonistic activity [11].

2-3-8- Determination of beta-galactosidase activity

First, 40 mg of X-gal (5-bromo-4-chloro-3-indolyl-beta-di-galactopyranoside) powder was dissolved in 2 ml of dimethylformamide. Then 60 microliters of this solution and 10 microliters of IPTG solution (iso-propyl-thio-beta-di-galactosidase) as an inducer on the surface of MRS solid medium and MRSC Prepared in advance, played. A colony of the mentioned bacteria was cultivated linearly and kept for 2 days at a temperature of 30°C, and this characteristic was checked through the color of the colony [10].

2-3-9-Determining the ability to adhere to human intestinal epithelial cells

HT-29 cell line, a human intestinal epithelial adenocarcinoma cell that secretes mucus, was purchased from Khayyam Biomanuvar Cell Culture Laboratory, Mashhad. These cells in RPMI 1640 culture medium[15] Contains 10% (v/v) fetal bovine serum[16] Inactivated by heat and standard mixture of antibiotics (penicillin, streptomycin) were grown at 37°C under 5% carbon dioxide pressure. Growth of HT-29 cells It continued in cell culture flasks for a week and its culture medium was changed every other

6. Disk diffusion
7. Mueller-Hinton agar
8. Gentamicin
9. Erythromycin
10. Amoxicillin
11. Tetracycline
12. Chloramphenicol
13. Vancomycin
14. Penicillin
15. Gibco Roswell Park Memorial Institute medium
16. Fetal bovine serum

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day. The adhesion test was performed in a 6-well plate with 250,000 cells/ml. After the formation of a thin layer of cells on the bottom of the plate, it was washed twice with phosphate buffered saline solution to remove the antibiotic, and one milliliter of the bacterial suspension obtained from the 18-hour culture of the strains with a concentration equal to half McFarland's was added to HT-29 cells. It was added and placed in a greenhouse for two hours at 37°C and 5% carbon dioxide. Then, in order to remove unattached cells, washing was done with phosphate buffer saline solution. HT-29 cells and attached bacteria using ethylenediamine-tetraacetic acid-trypsin solution[17] (Sigma-Aldrich) were separated and after serial dilution in MRS medium and MRSC Agar was counted. Bacterial adhesion ability was reported based on the number of attached bacteria compared to the total number of primary bacteria [12].

2-4- Statistical analysis

Tests in the form of randomized complete block design and at least in three repetitions using one-way analysis of variance and with the help of SPSS software. (USA, ed. 24) was done. Means were compared with Duncan's test method and all data analysis was done at 95% confidence level.

3. Results and Discussion

3-1- Resistance to bile acid and salt

One of the important criteria in assessing the potential of probiotic strains is their resistance to acidic conditions and high concentration of bile salts [13]. As seen in Table 1, both strains showed good tolerance at pH=3. bacteria*Bifidobacterium animalis* subspecies*Lactis*BB-12 at pH=2 With the lowest logarithmic decrease in number, the strain was resistant; If growth and survival*Lactobacillus plantarum*ATCC 14917 was strongly reduced at this pH. The resistance of both bacteria was almost the same after three hours at pH = 3 and they showed a high survival rate of 84%. The pH of the human stomach ranges from 1.5 to 4.5, but in most of the in vitro studies, pH=3 is used to evaluate probiotic properties, which is due to the very low survival of bacteria at pH=2. When probiotic strains are exposed to the extreme pH of the stomach, they become buffered by food or other molecular carriers [14]. On the other hand, this ability to tolerate acid has been attributed to the synthesis of various polysaccharide compounds that play a role in protecting the cell membrane [15]. According to the research of Jangersen et al. (2014)*Bifidobacterium animalis* subspecies*Lactis*BB-12 has a high survival rate in acidic pHs and this feature is due to the activity of the F complex$_0$F$_1$-ATPase has been attributed to be able to facilitate the release of hydrogen ions in acidic conditions and help maintain homeostasis inside the bacterial cell [16]. Stasiak-Rozanska et al. (2021) in the study of the viability of commercial probiotic strains in the pH range of the digestive tract in the food matrix found that the strain*Bifidobacterium animalis* subspecies*Lactis*BB-12 Able to survive above 80% pH 2 and 3 after the passage of at least one hour[17], which confirms the results of the present study; The results of this study were consistent with other previous studies [11, 16, 18].

Table 1 Acid and bile salt tolerance of strains

*Lactobacillus plants*ATCC 14917		*Bifidobacterium animalis subsp. Milk*BB-12			
Survival (%)	Viable count **	Survival (%)	Viable count**		
-	6.90 ± 0.01 [a]	-	7.06 ± 0.03 [a]	Initial count, t= 0 h	**Acid tolerance**
29.13	2.01 ± 0.07 [c]	93.48	6.60 ± 0.09 [b]	pH=2, t= 1 h	
89.13	6.15 ± 0.07 [b]	86.40	6.10 ± 0.02 [c]	pH=3, t= 1 h	
88.84	6.13 ± 0.01 [b]	84.99	6.00 ± 0.00 [d]	pH=3, t= 3 h	
-	8.17 ± 0.05 [a]	-	6.80 ± 0.01[a]	0%	**Bile salt tolerance**
99.14	8.10 ± 0.04 [a]	92.35	6.28 ± 0.03 [b]	0.3%	
92.66	7.57 ± 0.04 [b]	91.03	6.19 ± 0.02 [c]	0.5%	
74.42	6.08 ± 0.01 [c]	86.32	5.87 ± 0.04 [d]	1%	

*The values with different superscript letters in a same column are significantly different ($p<0.05$).
** The values are mean Log CFU/ml ±SD

[17]. Ethylenediaminetetraacetic acid-trypsin

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Bile salts are compounds derived from cholesterol that are synthesized in the liver and stored in the gallbladder as conjugated amino acids, and are secreted into the small intestine during the digestion process and help to emulsify and absorb lipids [19]. They are toxic to living cells in the cell membrane; Therefore, resistance to bile salts is one of the essential characteristics of lactic acid bacteria [20]. In this research, the concentrations of 0.3, 0.5 and 1% were used to check the resistance of the strains to bile salts. The results showed that both strains showed high resistance to bile. According to Table 1, the survival of the strains*Bifidobacterium animalis* subspecies*Lactis*BB-12 and*Lactobacillus plantarum*ATCC 14917 in the concentration of 0.3%, it was 92.35% and 99.14%, respectively; Also, the survival for both strains at concentrations of 0.5% and 1% was higher than 91% and 74%, respectively, which indicates the viability and activity of these two strains in the small intestine environment. The results showed that the strain*Bifidobacterium animalis* subspecies*Lactis*BB-12 is more resistant to changes in the concentration of bile salts, so that changes in the viability of the strains*Bifidobacterium animalis* subspecies*Lactis*BB-12 and*Lactobacillus plantarum*ATCC 14917 Between the concentration range of 0.3% and 1%, it was 6.03% and 24.72%, respectively. Due to their amphiphilic nature, bile salts destroy bacterial membranes and also exert oxidative stress on bacterial DNA. Cholylglycine hydrolase or bile salt hydrolase and oxalyl oxalate decomposing enzyme and decarboxylase enzyme A are the main effective enzymes in salt tolerance[19]. Existence of bile salt hydrolase enzyme gene and its activity in the strain*Bifidobacterium animalis* subspecies*Lactis*BB-12 makes it possible to quickly respond to high concentrations of bile salts and facilitate its passage through the small intestine and enter the large intestine [16]. Proteomic study results[18] and physiological of bile resistant strains in*Bifidobacterium animalis* subspecies*Lactis* showed that 5 pathways are involved in this resistance; (1) changes in the glycolytic pathway and energy production (2) changes related to nitrogen metabolism in which the sigmoid factor can be involved (3) changes in the biosynthesis of fatty acids (4) an increase in the amount of molecular chaperones and (5) changes in the redox balance cell[21]. Relatively high resistance of the strain*Lactobacillus plantarum*It is also probably related to the expression of bile resistance proteins in bacterial cells [6].

2-3- Determining the resistance in the simulated conditions of the digestive system

The passage of probiotics through the digestive system faces many challenges; The lysozyme enzyme found in saliva as the first defense barrier of the body is an antimicrobial protein that disrupts the cell wall of bacteria with its hydrolysis activity [22]. Exposure to high acidity and the presence of pepsin in the stomach is another obstacle for probiotics to pass through the digestive system. The daily secretion of 2.51 liters of gastric juice with a pH of about 2 and the antimicrobial activity of pepsin destroys most of the microorganisms digested with food. The presence of bile salts and pancreatin in the small intestine is the last challenge for probiotics to survive on the way through the digestive system. Therefore, most of the in vitro studies should pay attention to these things in order to select the probiotic strain [9, 23, 24]. According to Table 2, both strains were resistant to lysozyme (100 mg/liter); After 1 hour survival percentage*Lactobacillus plantarum*ATCC ١٤٩١٧*Bifidobacterium animalis* و و subspecies*Lactis*BB-12 was equal to 98.5 and 97.76 percent, respectively, which are in accordance with previous studies [11, 23]. The reason for the resistance*Lactic acid bacteria* The lysozyme enzyme has been attributed to the peptidoglycan structure of the cell wall, the physiological state of the cell and the concentration of the enzyme in the environment [25]. According to Table 2, the study of the survival of the desired strains in the conditions of gastric juice with pH = 3 after 3 hours showed that both strains are able to survive these conditions with a survival rate of more than 85%. Comparing the results obtained in this section with the acid resistance results showed that the strain*Lactobacillus plantarum*ATCC 14917 In the acidic environment with pH = 3, it shows relatively better survival, which is probably related to the proteins in MRS broth, which have a protective

[18]. Proteomic

effect on bacterial cells [6]. While strain*Bifidobacterium animalis* subspecies*Lactis*BB-12 shows relatively better resistance in the presence of pepsin, which is probably due to the role of pepsin in maintaining pH homeostasis and ATPase activity of the cell membrane [20, 26]. The survival rate of strains in the intestine for*Bifidobacterium animalis* subspecies*Lactis*BB ۱۲و-*Lactobacillus plantarum*ATCC 14917 It was equal to 86.94 and 90.83 percent, respectively. In general, both strains had a higher survival rate in the intestinal environment than in the stomach environment and resistance*Lactobacillus plantarum*ATCC 14917 It was higher in simulated stomach-intestinal conditions. The results obtained in this study were consistent with previous studies [7, 20, 27-29].

Table 2 Effect of simulated gastric (pepsin (3 mg ml^{-1}), NaCl (0.5% (w/v)) and pH=3) and intestinal (pancreatin (1 mg ml^{-1}), NaCl (0.5% (w/v)) and pH=8)fluids on viability of strains.

Simulated intestinal juice			Simulated gastric juice			Lysozyme resistance	
Survival (%)	Viable count (log (CFU/ml)), t= 4 h	Viable count (log (CFU/ml)), t= 0 h	Survival (%)	Viable count (log (CFU/ml)), t= 3 h	Viable count (log (CFU/ml)), t= 0 h	Survival (%)	**Strain**
86.94	4.64 ± 0.03	5.33 ± 0.03	85.10	4.49 ± 0.02	5.28 ± 0.02	97.76	*Bifidobacterium animalis subsp. Milk*BB-12
90.83	4.72 ± 0.04	5.20 ± 0.05	88.20	4.63 ± 0.03	5.26 ± 0.01	98.50	*Lactobacillus plants*ATCC 14917

3-3- Hydrophobicity, self-aggregation and beta-galactosidase activity

Cell surface characteristics measured by self-aggregation and hydrophobicity tests are important indicators for evaluating the adhesion capacity of probiotic cells to intestinal epithelial cells. Self-aggregation refers to the accumulation of bacterial cells of the same strain and is an important feature in biofilm formation and strain protection in gastrointestinal conditions and colonization in the intestine [30]. Self-assembly after 3 and 24 hours and hydrophobicity in the presence of n-hexadecane are shown in Figure 1. Self-accumulation of both strains after 3 hours was 15.84-18.29% and after 24 hours, 45.89-45.51% was obtained. Wang et al. (2010) stated that self-aggregation above 40% is favorable and strains with self-aggregation less than 10% have weak self-aggregation. The highest rate of self-accumulation in this study is in the strain*Bifidobacterium animalis* subspecies*Lactis*BB-12 belonged and grew over time. Water repellency*Bifidobacterium animalis* subspecies*Lactis*BB-12 59.75%*and Lactobacillus splentarum*ATCC was 42.1491749%. High hydrophobicity is related to bacterial cell surface glycoproteins and low hydrophobicity is related to bacterial cell surface polysaccharides. An important point to note is that the hydrophobicity potential is different among organisms and strains and depends on the age and surface chemistry of bacterial cells along with environmental components [13]. Both strains produced green colored colonies after 48 hours, which indicated the presence of beta-galactosidase enzyme. Beta-galactosidase hydrolyzes lactose into galactose and glucose and thus improves lactose intolerance in affected people. Similar reports by other researchers confirmed the results of self-healing, hydrophobicity and β-galactosidase activity [8, 11, 29, 31].

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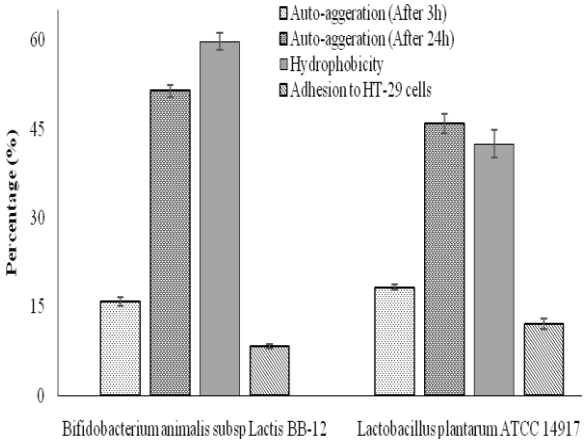

Fig 1 The auto-aggregation (after 3 and 24 h), hydrophobicity and adhesion ability of strains to HT-29 cell line.

3-4-antibiotic resistance

The normal flora of the intestine is mainly disturbed by the use of antibiotics, which leads to the abnormality of the intestine. The use of antibiotic-resistant strains preserves the natural intestinal bacterial flora [32]. Being safe is one of the important features

It is probiotic strains that part of this immunity is related to not having acquired and transmissible antibiotic resistance [31]. The results related to resistance to antibiotics are given in Table 3. Both strains*Bifidobacterium animalis* subspecies*Lactis*BB ۱۲و-*Lactobacillus plantarum*ATCC 14917 were resistant to penicillin, vancomycin (inhibitor of cell wall synthesis) and tetracycline (inhibitor of protein synthesis) and were sensitive to erythromycin, chloramphenicol (inhibitor of protein synthesis) and amoxicillin (inhibitor of cell wall synthesis).*Bifidobacterium animalis* subspecies*Lactis*BB-12 was also sensitive to gentamicin (a protein synthesis inhibitor). Vancomycin is one of the antibiotics prescribed in the treatment of multidrug-resistant pathogens, so resistance to this antibiotic is an important issue. In this study, both strains were resistant to this antibiotic. [25]. It also seems that resistance to gentamicin is inherent in Lactobacillus plantarum strains. In general, the strainHi*Lactobacillus* to aminoglycoside antibiotics (gentamicin, kanamycin[19]Neomycin[20] and streptomycin[21]) are resistant to beta-lactams (penicillin and ampicillin).[22]), antibiotics effective on gram positives (erythromycin and novobiocin[23]) and broad-spectrum antibiotics (chloramphenicol, erythromycin and rifampin[24]) are sensitive. A review of the sources showed that*Lactobacillus plantarum*It shows variable behavior towards penicillin and tetracycline antibiotics[33]. The results of this study were consistent with previous studies [33-36]. One of the reasons for resistance*Lactic acid bacteria* Antibiotics can be referred to protective mechanisms such as drug modification or inactivation, target site modification, metabolic pathway modification, and drug accumulation reduction [11, 37, 38].

Table 3 Antibiotic susceptibility of strains

Diameter of inhibition zone (mm)							Strains
Penicillin G	Vancomycin	Chloramphenicol	Tetracycline	Amoxicillin	Erythromycin	Gentamicin	
6.00± 0.00 [Its]	6.00± 0.00 [Its]	28.67± 1.24 [a]	13.66 ± 0.82[d]	25.50± 0.41[b]	25.50 ± 0.41 [b]	16.33 ± 1.24 [a]	*Bifidobacterium animalis subsp. Milk*BB-12
6.00± 0.00 [f]	6.00± 0.00 [f]	29.50 ± 0.40 [b]	11.00 ± 0.81[d]	28.17 ± 1.64 [c]	33.67 ± 1.24 [a]	11.17 ± 1.02[Its]	*Lactobacillus plants*ATCC 1491

*The values with different superscript letters in a same column are significantly different ($p<0.05$).
**Gentamycin results based on R ≤ 12 mm; I: 13–15 mm; S ≥ 16 mm. Erythromycin results based on R ≤ 13 mm; I:13–23 mm; S ≥ 23 mm. Tetracycline results based on R≤ 14 mm; I: 15–18 mm; S ≥ 19 mm. Vancomycin results based on R ≤ 12 mm; I: 12–13 mm; S ≥ 13 mm.R: resistant (zone diameter, ≤12.4 mm); I:intermediate (zone diameter, 12.5–17.4 mm); S: susceptible (zone diameter, ≥17.5).

19. Kanamycin
20. Neomycin
21. Streptomycin
22. Ampicillin
23. Novobiocin
24. Rifampin

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5-3- Ability to adhere to human intestinal epithelial cells

Adherence and temporary colonization of probiotics in the intestine are important features for creating health-enhancing effects in the host [39]. As shown in Figure 1, the percentage of adhesion to HT-29 cells for*Lactobacillus plantarum*ATCC 14917 and*Bifidobacterium animalis* subspecies*Lactis*BB-12 was equal to 12.14 and 8.35 percent, respectively. According to the study of Zhang et al. (2020), the adhesion of strains*Lactobacillus plantarum*The maximum was 19%, which was consistent with the present study [29, 40]. In this study, the amount of adhesion obtained for*Bifidobacterium animalis* subspecies*Lactis*BB-12 was more than the amount reported in the study of Arbolia et al. (2011) [7]. Although hydrophobicity and self-accumulation are among the evaluation indicators in the selection of probiotic strains, they do not necessarily have a direct relationship with the adhesion of the strains to the intestinal epithelial cells [39]. Cell adhesion mechanism*Lactic acid bacteria* It is not yet clearly defined, but reports indicate that adhesion is a multifactorial mechanism that includes hydrophobic, stearic, and electrostatic interactions and specific structures such as elongation factor EF-Tu, chaperonin Gro-EL and includes DnaK chaperone[30].

4 - Conclusion

In this research, the probiotic characteristics of two commercial strains *Bifidobacterium animalis* subspecies *Lactis*BB-12 and *Lactobacillus plantarum* ATCC 14917 was investigated in vitro. Both strains were able to grow and survive in the presence of acid and bile, and also showed a high survival rate in simulated conditions of the stomach and intestine. Both strains had beta-galactosidase enzyme, showed high self-aggregation and hydrophobicity and were able to adhere to intestinal epithelial cells. The results of evaluating the better performance of the strain *Lactobacillus plantarum* ATCC 14917 showed in similar conditions of the digestive system.

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مقاله علمی_پژوهشی

ارزیابی ویژگی‌های پروبیوتیکی سویه‌های تجاری *لاکتوباسیلوسپلانتاروم* و *بیفیدوباکتریومانیمالیس* زیرگونه*لاکتیس*در شرایط برون‌تنی

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چکیده

پروبیوتیک‌ها میکروارگانیسم‌های زنده‌ای هستند که در صورت مصرف به میزان کافی، تأثیرات سودمندی بر سلامت میزبان خواهند داشت. در پژوهش حاضر خصوصیات پروبیوتیکی دو سویه تجاری *بیفیدوباکتریومانیمالیس* زیرگونه *لاکتیس* BB-12 و *لاکتوباسیلوسپلانتاروم* ATCC 14917 در شرایط برون‌تنی مورد بررسی قرار گرفت. نتایج نشان داد که هر دو سویه مقاومت بالایی در برابر اسید، صفرا و لیزوزیم داشتند. به‌طورکلی میزان زنده‌مانی هر دو سویه در شرایط شبیه‌سازی شده معده-روده‌ای بالاتر از ۸۵٪ بود که امکان زنده‌مانی این دو سویه در دستگاه گوارش را فراهم می‌سازد. به‌علاوه بالاترین میزان آب‌گریزی (۵۹/۷۵٪) و خودانبوهش (۵۱/٤۲٪) و همچنین کمترین چسبندگی (۸/۳۵٪) به رده سلولی HT-29 روده‌ی انسان مربوط به *بیفیدوباکتریومانیمالیس* زیرگونه *لاکتیس* BB-12 بود؛ هر دو سویه دارای فعالیت بتا گالاکتوزیدازی بودند و نسبت به آنتی‌بیوتیک‌های پنی‌سیلین، ونکومایسین و تتراسایکلین مقاومت نشان دادند. در پژوهش حاضر مشخص گردید که *لاکتوباسیلوسپلانتاروم* ATCC 14917 نسبت به *بیفیدوباکتریومانیمالیس* زیرگونه*لاکتیس* BB-12 خصوصیات پروبیوتیکی بهتری دارد.