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Adhesion Properties of Probiotic *Lactobacillus* Strains Isolated from Tunisian Sheep and Goat Milk

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ABSTRACT

Four hundred strains isolated from Tunisian sheep and goat raw milks were initially screened for their ability to survive the GastroIntestinal Tract (GIT). Forty-three among the four hundred bacteria were resistant to pepsin, pH 2, pancreatin and bile salts at 0.3%, even after 5 hours of incubation. Identification using *16S rRNA* gene sequencing was established and we obtained as a species *Lactobacillus plantarum* (29 isolates from sheep milk and 11 from goat milk) and *Lactobacillus pentosus* (2 isolates from sheep milk and 1 from goat milk). We showed the ability for auto-aggregation and/or hydrophobicity properties. Finally, both M63 and C78 strains showed an important level of adhesion to three intestinal epithelial cells Caco-2 TC7, HT29-MTX, and HT29-CL.16E. Taken together, these properties allow the lactobacilli strains to be considered promising beneficial strains for developing functional foods for consumers.

Keywords: Auto-aggregation, Functional foods, Hydrophobicity, Lactobacilli.

INTRODUCTION

The importance of milk in human nutrition is well established and its regular consumption is recommended. In recent years, the importance of sheep and goat milks to human health have been increasingly demonstrated (Kapila *et al*., 2013) and their nutritional and health benefits have been related to a number of certain diseases of people such as the allergy to food with bovine milk proteins (Yuksel *et al*., 2012).

The surplus of sheep and goat milks is well observed in Tunisia, especially in the northeast and semi-arid regions during the months of high production (Mohamed *et al*., 2008).

In fact, the first aspect to demand for sheep and goat milks and their derivatives were the consumer interest in functional foods and gain access to the market. These two milks have important biological properties. They may contain different elements of nutritional or medicinal importance and they are associated with low microbiological and technological qualities (Miedico *et al*., 2016). For the second aspect, cheeses and yoghurts manufactured with these milks were recognized by consumers as gastronomic products higher than those with bovine milk (Milani and Wendorff, 2011).

According to the definition adopted by the FAO/WHO (2002), probiotics are living microorganisms that confer a health benefit on the host, when administered in adequate amounts.

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The majority of the probiotics belong to the genus *Lactobacillus* and *Bifidobacterium*. Even though some species of these genera are "Generally Recognized As Safe" (GRAS) by the United States Food and Drug Administration (FAO/WHO, 2002). It was recommended for further studies to demonstrate if a probiotic strain is safe. In order to act as a probiotic, bacteria must survive the acidic conditions of the stomach, tolerate the presence of pancreatic enzymes, and resist the bile acids in the GastroIntestinal Tract (GIT) (Rönkä *et al*., 2003). Lactic acid bacteria are considered natural inhabitants of the GIT, thus intestinal bacteria should be selected for use as probiotics (Vizoso-Pinto *et al*., 2006). Autoaggregation and hyrophobicity of probiotics may influence the adhesion ability of the bacteria to intestinal epithelial cells (Kotzamanidis *et al*., 2010).

For beneficial health effects on humans, probiotics alleviate the intolerance of lactose, enhance the nutrients bioavailability and prevent or reduce the allergies. They have anticarcinogenic, hypocholesterolemic, antihypertensive, antiosteoporosis, and immunomodulatory effects (Chiang and Pan, 2012; Ardalanian and Fadaei, 2018).

Therefore, the objective of this research was to identify and evaluate the probiotic properties of strains isolated from several samples of Tunisian sheep and goat milks. Probiotic properties would be evaluated by tolerance to pepsin at pH 2, pancreatin and bile salts (0.3%), surfaces properties (aggregation and hydrophobicity) and adhesion assays employing Caco-2 TC7, HT29-MTX and HT29 CL.16E epithelial cell lines.

MATERIALS AND METHODS

Sample Collection and Bacterial Isolation

One hundred sixty samples of sheep and goat milks were aseptically collected from different regions of Tunisia (North, Northwest, Northeast, Coast, Midle and South) and stored at 4°C. Four hundred strains were isolated by the dilution plate method on specific medium Man Ragosa Sharpe (MRS) (Biokar Diagnostics, Solabia, France).

Resistance to Biological Barriers

Following the method of Pennacchia *et al*. (2004), bacterial cells from overnight cultures (37°C, 24 hours) were harvested (10,000×g, 5 minutes, 4°C) (Centrifuge, Boeco, Hamburg, Germany) and washed twice with Phosphate Buffered Saline (PBS) (Sigma Aldrich, Saint-Quentin-Fallavier, France) at pH 7, before being resuspended either in PBS (pH 2) or in pepsin (0.3%, Sigma Aldrich, Saint-Quentin-Fallavier, France). Dilution series were prepared from inoculums that were already incubated at 37°C for 3 hours and viable colonies were enumerated on MRS agar. The strains resistant to pepsin at pH 2 were further tested for their resistance to pancreatin in PBS solution at pH 8, containing pancreatin (3 g L^{-1}, Sigma Aldrich, Saint-Quentin-Fallavier, France). Viable colonies were enumerated after incubation of inoculums at 37°C for 3 hours. Survival rates were calculated according to the following equation:

$$Survival\ rate\ (\%) = (N_1/N_0) \times 100$$

Where, N_1 represents the total viable count of strains at time 3 «or» 4 hours and N_0 represents the total viable count of strains at time 0 hour.

Bile salt tolerance was tested according to Anandharaj and Sivasankari (2014) with modifications. Overnight cultures were inoculated in MRS broth (Biokar Diagnostics, Solabia, France) containing bile salts (Oxgall, Sigma Aldrich, Saint-Quentin-Fallavier, France) at 3 g L^{-1}. The mixture was incubated at 37°C for 5 hours and viable count was determined by the plate method.

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Hemolytic Test

This test was determined by spotting on blood agar plates according to Meira *et al*. (2012). This activity may be found in the appearance of a clear halo around the colony (β-hemolysis), a green halo (α-hemolysis) or it may not be detected (γ-hemolysis).

Identification of Isolates

Forty-three selected strains were identified using 16S rRNA sequence analysis according to the method adopted in URAFPA (Research Unit and Animal Products Features - University of Lorraine – INRA – Nancy- France). *16S rRNA* genes were amplified by PCR using the universal primer SSU for: TGCCAGCAGCCGCGGTA and SSU rev: *GACGGGCGGTGTGTACAA* (Eurogentec, Serain, Belgium). Amplification was determined using a Mastercycler pro thermocycler (Eppendorf, Hambourg, Germany). as follows: denaturation at 95°C for 3 minutes, 35 cycles of 30 seconds at 95°C, 30 seconds at 55°C, 1 minute at 72°C, 10 minutes at 72°C. Amplified fragments were purified from agarose gels by a High Pure PCR purification kit (Roche (Roche Applied Science, Meylan, France). The analysis of alignment and homology of the partial nucleotide sequence of *Lactobacillus* spp. was determined by the basic local alignment search tool (Bioedit and BLAST NCBI, Beckman Coulter Genomics, Nancy, France).

Hydrophobicity and Autoaggregation Tests

Following the method of Thapa *et al*. (2004), bacterial cells were harvested (6,000×g, 4°C, 5 minutes), once washed and resuspended in Ringer's solution (10 mL) (Jeulin, Marseille, France) and OD_{600} of aqueous phase was measured (Spectrophotometer, Boeco, Hamburg, Germany). Then, 10 mL of xylene (Jeulin, Marseille, France) was added to the cell suspension and the mixture was well vortexed for 2 minutes (Vortex, Boeco, Hamburg, Germany). After 30 minutes, the two phases were separated and OD_{600} of non-aqueous phase was measured. The adhesion of strains to the xylene was previously determined Patel *et al*. (2009).

Each overnight culture was harvested (6,000×g, 4°C, 5 minutes) according to Kos *et al*. (2003), washed twice with PBS (pH 7.3) and resuspended in PBS to obtain OD_{595}= 0.5. Four mL of each cell suspension was vortexed for 10 seconds and incubated at 37°C for 1 minute. OD_{595} of the upper layer was determined. The percentage of aggregation was previously expressed by Patel *et al*. (2009).

Antimicrobial Activity towards Pathogens

The antagonism towards pathogens such as *Staphylococcus aureus* (ATCC 25923), *Listeria monocytogenes* (ATCC 070 101 121), *Salmonella thyphimirium* (ATCC 25922) and *Escherchia coli* (DH5 alpha, Institute Pasteur of Tunisia) was evaluated according to Villani *et al*. (1997) and Ben Moussa *et al*. (2008) with some modifications. Each overnight culture was spotted on MRS agar and incubated at 37°C for 24 hours. 100 µL of an overnight culture of four indicator pathogens was inoculated in 5 mm of soft nutrient agar (Biokar Diagnostics) and was spread on the spotted strains followed again by incubation at 37°C for 24 hours (Mahmoudi *et al*., 2016).

Growth and Maintenance of Cell Lines

Three different intestinal epithelial cells were used for adhesion tests. The Caco-2 TC7 were obtained from Pr. Isabelle Chevalot and the HT29-MTX were kindly provided by Dr. Thécla Lesuffleur (1990) (Mahmoudi *et al*., 2016). The HT29-CL.16E

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cells are derived from the human colonic cancer HT29. After sodium butyrate adaptation, they have acquired the ability to differentiate into polarized goblet cells (Augeron and Laboisse, 1984). They were obtained from Ephyscience, Nantes, France.

The growth conditions of Caco-2 TC7, HT29-MTX and HT29-CL.16E cells were described elsewhere by Kebouchi *et al*. (2016).

Adhesion Assay

Adherence capacity to different cells was tested according to Turpin *et al*. (2012) and Kebouchi *et al*. (2016). The cells were seeded at a concentration of 5 10^6 cells $well^{-1}$ in six well culture plates for 21-25 days for using in adhesion test. The bacterial cultures (14 h, 20 mL) were harvested (4,000×g, 15 minutes, 4°C) and washed with Hank's Balanced Salt Solution (HBSS) (Fisher Scientific, Invitrogen, Boulevard Sébastien Brant, France). Then, cells were resuspended in Dulbecco's modified Eagle's Minimal Essential Medium (DMEM) (Fisher Scientific, Invitrogen, Boulevard Sébastien Brant, France) without antibiotics to obtain a concentration of 10^9 CFU mL^{-1}. The growth medium in the six-well culture plate's monolayers was eliminated by aspiration and the cells were washed once with HBSS. A 2-mL aliquot of cell suspension (10^9 CFU mL^{-1} in DMEM) was added to each well of the tissue culture plate (to obtain bacterial cell to epithelial cell ratio of 1,000: 1) and incubated at 37°C in 10% CO_2 atmosphere for 2 hours. Therefore, the supernatants from each well were recovered and the cell monolayers were washed four times with HBSS to recuperate non-adherent bacterial suspensions. *Streptococcus thermophilus* strain LMD-9 (ATCC® BAA-491™; GenBank accession: CP000419), originally isolated from yogurt, was used in this study as control (Kebouchi *et al.* 2016). The number of adherent bacterial cells from the cell pellet and non-adherent bacterial cells from the supernatants and washing solutions were determined by plating serial dilutions on LM17 agar or MRS agar plates, and colonies were counted by visual counting after 24–48 hours of incubation in anaerobic conditions (AnaeroGen, Oxoid, Basingstoke, UK).

Statistical Analysis

All numerical data were performed by one-way analysis of variance ANOVA followed by Student's test. The results were shown as the mean±standard deviation of three independent repetitions. A *P* value below 0.05 was considered statistically significant.

RESULTS

Selection and Identification of Strains

The results obtained by exposure of isolates to pH 2, pepsin, pancreatin and 0.3% of bile salts are reported in Table 1. Thirty-one strains isolated from sheep milk and twelve strains isolated from goat milk were resistant out of four hundred isolates. The counts of all the strains were beyond < 0.1 log CFU mL^{-1}. No significant differences were shown in the viability of the strains (P> 0.05). In response to pancreatin, similar data were reported for all tested strains and their decreasing viability was lower than 0.1 log CFU mL^{-1} (Table 1).

Also, all the strains were growing in 0.3% oxgall after 5 hours of exposure. Both strains M63 and C78 grew better than the rest of the strains with a decrease lower than 4.5 log CFU mL^{-1}. For safety aspect, all the selected strains were γ-hemolytic.

16S rRNA gene sequences were determined for the selected strains. Forty strains belonged to *Lactobacillus plantarum* and only two isolated from sheep's milk (M67 and M70) and one isolated from goat milk (C2) belonged to *Lactobacillus pentosus* (Table 1).

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Table 1. Resistance of forty-three isolates to gastrointestinal stress.

Strains	Origin	Gastrointestinal conditions			Species
		Pepsin[c]	Pancreatin[d]	Bile salt[e]	
M4	Sm[a]	-0.03±0.01	-0.02±0.001	-5.42±0.02	*L. plantarum*
M29	Sm	-0.02±0.11	-0.02±0.001	-5.89±0.28	*L. plantarum*
M46	Sm	-0.06±0.02	-0.02±0.03	-5.19±0.013	*L. plantarum*
M54	Sm	-0.07±0.03	-0.02±0.08	-4.5±0.004	*L. plantarum*
M63	Sm	-0.01±0.01	-0.01±0.01	-4.23±0.005	*L. plantarum*
M67	Sm	-0.07±0.01	-0.05±0.01	-6.12±0.1	*L. pentosus*
M70	Sm	-0.02±0.07	-0.06±0.13	-6.64±0.1	*L. pentosus*
M74	Sm	-0.07±0.14	-0.02±0.01	-5.20±0.5	*L. plantarum*
M85	Sm	-0.05±0.02	-0.02±0.04	-5.66±0.01	*L. plantarum*
M97	Sm	-0.05±0.11	-0.02±0.01	-5..48±0.01	*L. plantarum*
M104	Sm	-0.11±0.05	-0.02±0.03	-5.44±0.01	*L. plantarum*
M109	Sm	-0.08±0.01	-0.02±0.01	-5.75±0.1	*L. plantarum*
M113	Sm	-0.05±0.009	-0.02±0.01	-5.06±0.01	*L. plantarum*
M118	Sm	-0.08±0.01	-0.02±0.002	-5.08±0.01	*L. plantarum*
M129	Sm	-0.09±0.01	-0.02±0.01	-4.74±0.1	*L. plantarum*
M140	Sm	-0.02±0.01	-0.02±0.21	-4.52±0.1	*L. plantarum*
M146	Sm	-0.01±0.09	-0.06±0.01	-4.55±0.19	*L. plantarum*
M180	Sm	-0.09±0.17	-0.07±0.01	-4.85±0.01	*L. plantarum*
M181	Sm	-0.03±0.01	-0.08±0.01	-4.71±0.01	*L. plantarum*
M186	Sm	-0.08±0.01	-0.09±0.01	-4.86±0.01	*L. plantarum*
M192	Sm	-0.05±0.32	-0.02±0.01	-5±0.01	*L. plantarum*
M195	Sm	-0.02±0.05	-0.011±0.01	-4.89±0.01	*L. plantarum*
M199	Sm	-0.07±0.01	-0.012±0.01	-5.14±0.31	*L. plantarum*
M207	Sm	-0.04±0.25	-0.013±0.01	-5.62±0.01	*L. plantarum*
M211	Sm	-0.09±0.01	-0.02±0.01	-4.99±0.1	*L. plantarum*
M215	Sm	-0.03±0.06	-0.02±0.02	-5.012±0.01	*L. plantarum*
M216	Sm	-0.03±0.01	-0.02±0.01	-5.33±0.1	*L. plantarum*
M217	Sm	-0.04±0.03	-0.02±0.009	-5.49±0.01	*L. plantarum*
M219	Sm	-0.03±0.01	-0.02±0.01	-4.55±0.1	*L. plantarum*
M220	Sm	-0.04±0.05	-0.02±0.07	-4.32±0.01	*L. plantarum*
M252	Sm	-0.05±0.01	-0.02±0.01	-5.02±0.5	*L. plantarum*
C2	Gm[b]	-0.01±0.16	-0.021±0.012	-5.63±0.01	*L. pentosus*
C11	Gm	-0.07±0.01	-0.02±0.01	-4.8±0.01	*L. plantarum*
C22	Gm	-0.08±0.01	-0.02±0.01	-5.2±0.01	*L. plantarum*
C27	Gm	-0.09±0.01	-0.02±0.01	-4.87±0.01	*L. plantarum*
C46	Gm	-0.04±0.21	-0.02±0.01	-5.26±0.01	*L. plantarum*
C51	Gm	-0.03±0.1	-0.02±0.01	-4.44±0.1	*L. plantarum*
C64	Gm	-0.02±0.01	-0.02±0.011	-4.89±0.27	*L. plantarum*
C78	Gm	-0.01±0.01	-0.01±0.01	-3.88±0.01	*L. plantarum*
C84	Gm	-0.04±0.2	-0.029±0.01	-4.66±0.13	*L. plantarum*
C87	Gm	-0.03±0.17	-0.03±0.08	-4.79±0.01	*L. plantarum*
C90	Gm	-0.03±0.01	-0.02±0.01	-4.45±0.01	*L. plantarum*
C96	Gm	-0.03±0.01	-0.02±0.001	-4.82±0.11	*L. plantarum*

[a] Sheep mil; [b] Goat milk; [c] Decrease of the counts (log CFU mL^{-1}) after 3 hours of incubation in pepsin solution at pH 2; [d] Decrease of the counts (log CFU mL^{-1}) after 4 hours of incubation in pancreatin fluid at pH 8.[e] Decrease of the counts (log CFU mL^{-1}) after 5 hours of incubation with 0.3% oxgall.

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Hydrophobicity and Autoaggregation Properties

The hydrophobicity percentages of strains isolated from sheep milk, using xylene as organic solvent, are presented in (Figure 1-a). The highest results belonged to strains M46 (15.33±0.57%), M211 (12.66±0.5%) and M216 (11.33±1.15%). In contrast, the strain M63 had the maximum level of auto-aggregation (57.33±4%) (Figure 1-b). Regarding the goat milk, the strain C78 had the lowest hydrophobicity compared to the other strains (Figure 2-a), but it had the important auto-aggregation with data of 60.7±1.1% (Figure 2b).

Antagonistic Activity

The data of antibacterial activity of forty-three strains towards four pathogens were regrouped in Table 2. All lactobacilli strains inhibited the growth of *S. aureus* and *L. monocytogenes*. Whereas, only the strains M63, M97, M104 and C78 inhibited the growth of *S. thiphimirium* and *E. coli*, the highest activities against *S. aureus* were observed with both M63 and C78, with inhibition zone diameter of 8±0.66 mm and 7.4±0.11 mm, respectively.

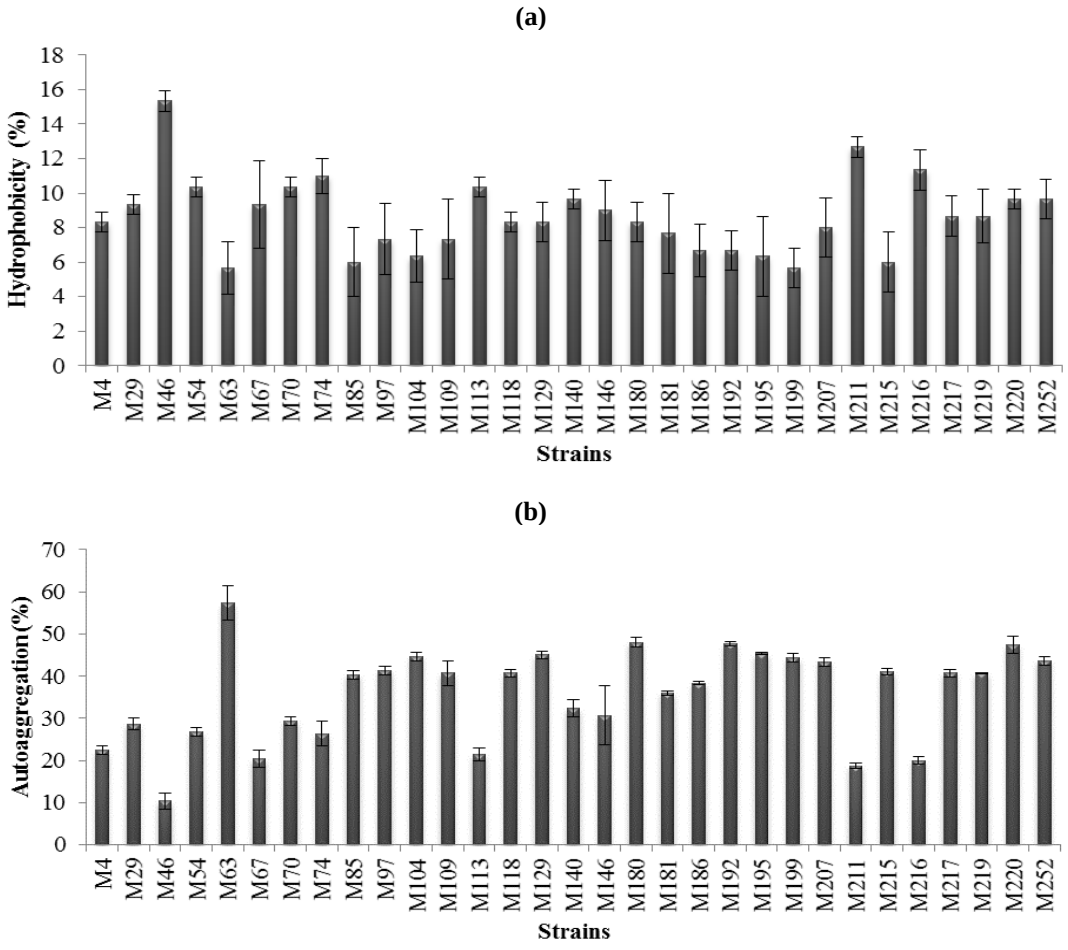

Figure 1. Percentage of hydrophobicity (a) and auto-aggregation (b) of lactobacilli strains isolated from sheep milk.

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Table 2. Antagonistic activity of forty-three isolates towards four indicator pathogens**.**

Strains	Antibacterial activity				
	L. monocytogenes	*S. aureus*	*S. thyphimirium*	*E. coli*	Addition
M4	+ [a]	++	-	-	3 +
M29	+	+	-	-	2 +
M46	+	++	-	-	3 +
M54	+	++	-	-	5 +
M63	++ [b]	++	+	+	6 +
M67	+	+	-	-	2 +
M70	+	+	-	-	2 +
M74	+	+	-	-	2 +
M85	+	++	-	-	3 +
M97	+	++	+	+	5 +
M104	+	++	+	+	5 +
M109	+	++	-	-	3 +
M113	+	++	-	-	3 +
M118	+	++	-	-	3 +
M129	+	++	-	-	3 +
M140	+	++	-	-	4 +
M146	+	++	-	-	3 +
M180	+	++	-	-	3 +
M181	+	++	-	-	3 +
M186	+	++	-	-	3 +
M192	+	++	-	-	3 +
M195	+	++	-	-	3 +
M199	+	++	-	-	3 +
M207	+	++	-	-	3 +
M211	+	++	-	-	3 +
M215	+	++	-	-	3 +
M216	+	++	-	-	3 +
M217	+	++	-	-	3 +
M219	+	++	-	-	4 +
M220	+	++	-	-	4 +
M252	+	++	-	-	3 +
C2	+	+	-	-	3 +
C11	+	++	-	-	3 +
C22	+	++	-	-	3 +
C27	+	++	-	-	3 +
C46	+	++	-	-	3 +
C51	+	++	-	-	3 +
C64	+	++	-	-	3 +
C78	++	++	+	+	6 +
C84	+	++	-	-	3 +
C87	+	++	-	-	3 +
C90	+	++	-	-	3 +
C96	+	++	-	-	3 +

[a] Presence of a clear zone of growth inhibition around spots ≤ 2 mm; [b] ++: Presence of a clearly defined inhibition zone between 2 and 8 mm; [c] +++: Presence of a clearly defined inhibition zone between 8 and 12 mm, and (–)= No inhibition.

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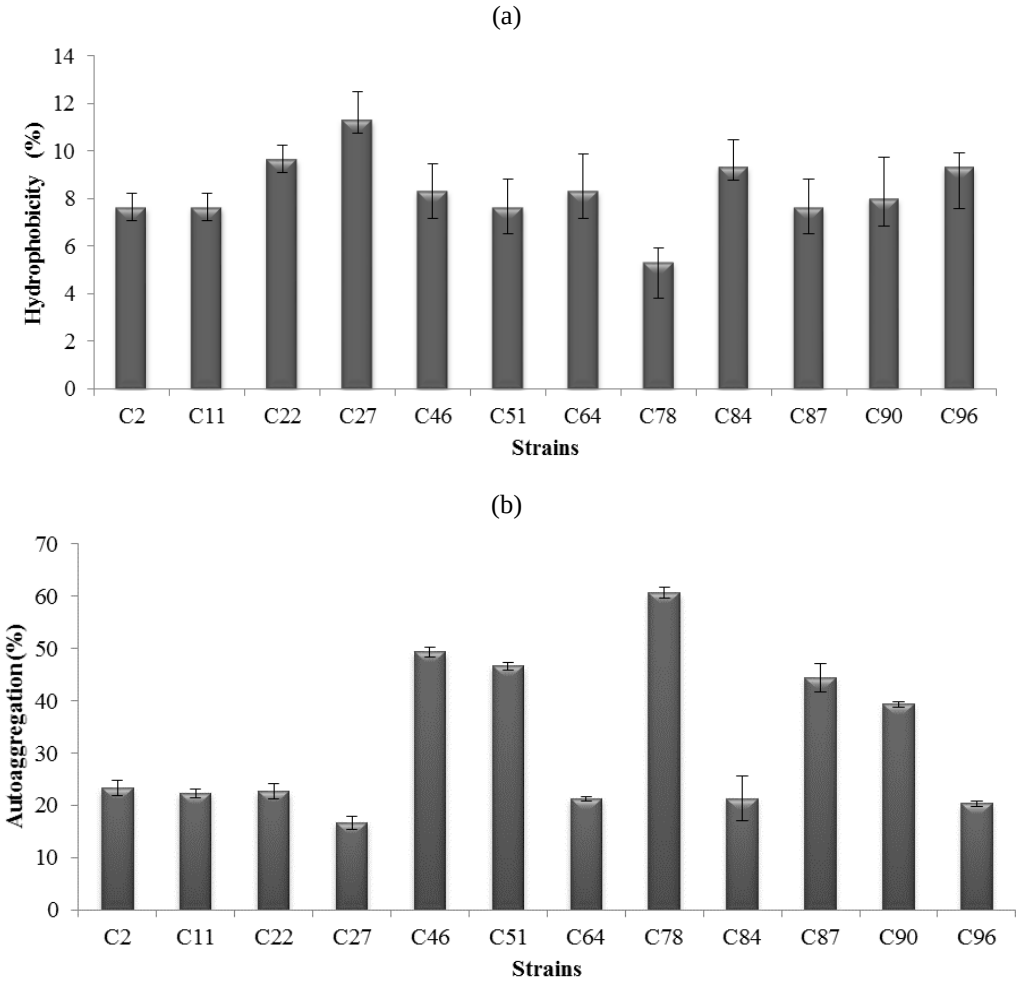

Figure 2. Percentage of hydrophobicity (a) and auto-aggregation (b) of lactobacilli strains isolated from goat milk.

Adhesion to Cell Lines

Both M63 and C78 strains have remarkable probiotic properties such as tolerance to bile salts at 0.3%, appreciated auto-aggregation, and important antibacterial activities against Gram positive pathogens, thus they were selected for adhesion assay to three epithelial intestinal cells.

The percentages of the adherence to Caco-2 TC7 for the two strains are presented in (Figure 3-a). Significant difference was demonstrated between M63 and C78, which adhered with values of 10.32±0.05 and 3.78±0.14%, respectively. We showed again a significant difference between strains and LMD-9 (0.41±0.2%). For HT29-MTX cell line, (Figure 3-b) shows that percentages ranged from 0.9±0.0 to 9.34±0.48%. We observed similar behavior in adherence of both strains to HT29-CL.16E with percentages of 97.29±1.43 and 88.5±8.6% for C78 and C63, respectively (Figure 3-c).

DISCUSSION

Tolerance to pepsin, low pH, pancreatic enzymes, and bile salts are studied to predict

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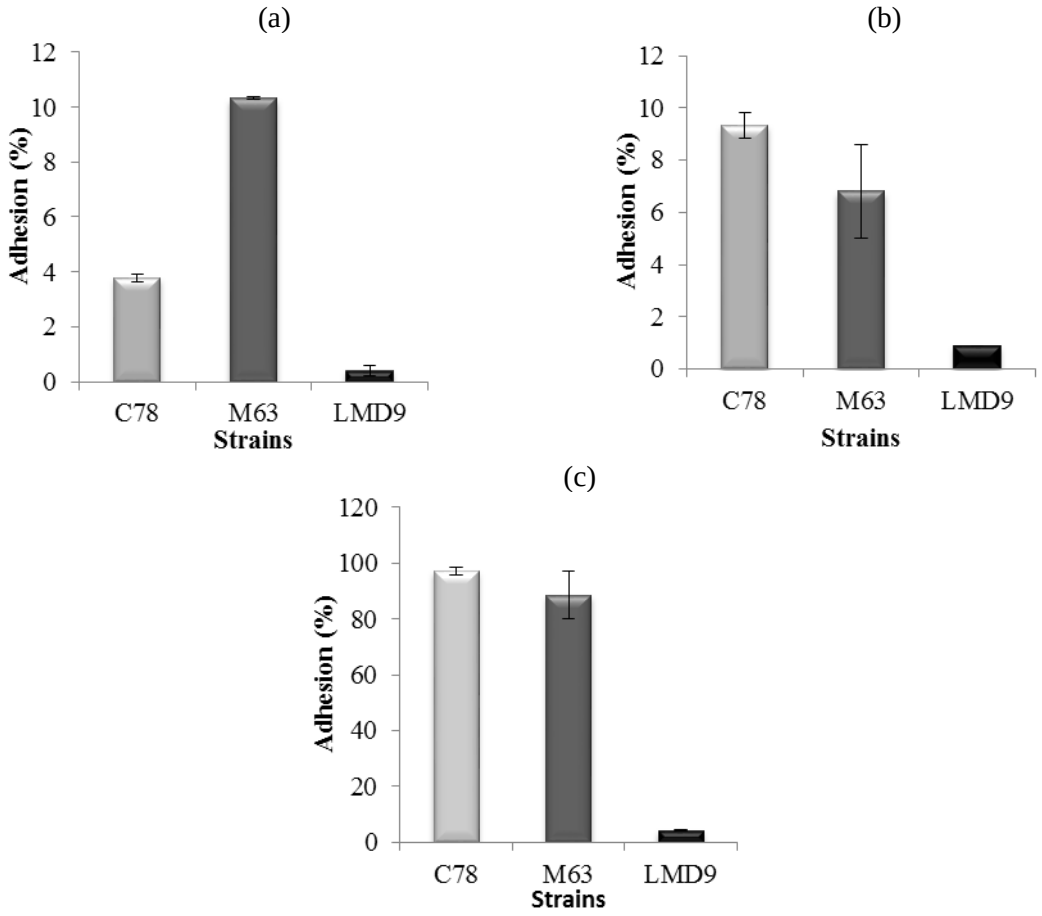

Figure 3. Adhesion ability to Caco-2 TC7 (A), HT29-MTX (B), and HT29-CL.16E (C) epithelial cell lines of two lactobacilli strains.

the survival of bacteria in the gastrointestinal conditions. In our study, when confronted with GIT conditions, all tested strains showed a decrease in viable counts less than 1 log CFU mL^{-1} with survival rate ≥ 90%, with no significant difference between those. Almeida Júnior *et al*. (2015), testing 50 strains isolated from goat milk, found that 36 strains were resistant to pH 2 with a survival rate ranging from 70 to 89%. Kumar and Kumar (2015) selected five isolates which were acid resistant with 60.52% of survival rate at pH 3. Moreover, Argyri *et al*. (2013) found that the majority of strains isolated from fermented olive showed a higher resistance to low pH. The pH 2 and lethal action of pepsin used in this work for the selection of probiotic strains are very selective (Pennacchia *et al*., 2004). Our results are also in agreement with those from previous research, where strains were isolated from food, human, or animal origins. We were able to conserve their viability when they were exposed to pH (2-4) (Vijayakumar *et al*., 2015). Regarding resistance to pancreatin, we have reported no significant action on the viability of the strains. Bile plays a fundamental role in defense mechanism of the gut and the behavior of its inhibitory effect is determined by the bile salt concentration (Charteris *et al*., 2000). Therefore, bile tolerance is considered as an important characteristic of probiotic strains, which enables them to survive in gastrointestinal transit. The tested strains were found to be resistant to bile salts (0.3%) even after 5 h of incubation, reducing their viability no lower than 6.64 log CFU mL^{-1}. In the work of Argyri *et al*. (2013), the majority of strains were

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resistant to bile salts, with a negligible reduction in viable counts lower than 1 log CFU g^{-1}. Mahmoudi *et al*. (2016) and Anandharaj and Sivasankari (2014) reported that all tested strains isolated from camel and mother milks, respectively, were resistant to bile salts at 1 % after 5 h of incubation. Sanders *et al*. (1996) reported that strains that could grow in normal physical bile concentration could survive in gastrointestinal transit. Absence of hemolytic activity is considered as a safety prerequisite for the selection of a probiotic strain (FAO/WHO, 2002). None of the examined strains exhibited hemolytic activity when grown on blood agar medium.

Two species were identified in the present study: *L. plantarum* and *L. pentosus*. The species *L. plantarum* was found in many research and was frequently isolated from different sources (Argyri *et al*., 2013; Anandharaj and Sivasankari, 2014; Vijayakumar *et al*., 2015; Mahmoudi *et al*., 2016; Mejri and Hassouna, 2016). *L. pentosus* was identified among strains isolated from fermented olive with important potential probiotic (Argyri *et al*., 2013).

Auto-aggregation and hydrophobicity of probiotic strains appeared to be necessary for adhesion to intestinal epithelial cells (Collado *et al*., 2007). However, the lactobacilli strains showed acceptable hydrophobic phenotypes and auto-aggregation pattern. Both of the strains M63 and C78 exhibited the highest auto-aggregation percentages. As concerns hydrophobicity, these isolates presented the lowest capacities to adhere to xylene as an apolar solvent. Many authors have proposed that these surface properties correlate with adherence capacity of probiotic strain (Kotzamanidis *et al*., 2010). Thus, the hydrophobicity character can indicate the attachment (electrostatic bonds and/or hydrogen) of different substrates that explain the probiotic property of bacteria by adhesion to organic solvents (Bellon-Fontaine *et al*., 1996). Moreover, adherent strains can inhibit the colonization of pathogens and establish a competition in the gastrointestinal system (Alander *et al*., 1997). Other conflicting evidences have been previously reported (Schillinger *et al*., 2005), suggesting that aggregative phenotype is a complex interplay of several factors other than hydrophobic ones, including passive forces, lipoteichoic acids and lectins, soluble and secreted proteins.

The concern about human pathogens is a major issue in the world. In the present study, antibacterial activity of 43 strains was tested against four pathogens. All strains inhibited both *L.monocytogenes* and *S.aureus;* and only four strains of *L. plantarum* were able to inhibit two pathogen strains as Gram negative. Argyri *et al*. (2013) found that none of the strains inhibited the tested pathogens. Comparing *L. acidophilus* strains isolated from infant faeces, they had weak antibacterial activity against *E. coli* (Xanthopoulos *et al*., 2000). In another study, Garriga *et al*. (1998) reported inhibition of *E. coli* by *Lactobacillus paracasei* subsp. *paracasei*. Daeschel (1989) reported that the antimicrobial effect of bacteria is due to the lactic acid, acetic acid, diacetyl, fatty acids, and other compounds.

The ability to adhere to the mucosal surfaces of the intestine has long been one of the most commonly criterion for the selection of probiotic strains. Despite sophisticated methodologies, adhesion capacity is mostly studied in *vitro* with epithelial cell lines or immobilized intestinal mucus (Jensen *et al*., 2012). We studied the adhesion capacity of the two strains M63 and C78 to the two models such as Caco-2, HT29-MTX, and to the best of our knowledge, the research of Kebouchi *et al*. (2016) is the first using the HT29-CL.16E cells for studying the bacterial adhesion. The percentages of adhesion of the strains were important and ranged from 3.78 % to 97.29 %. The higher adherence was found to HT29-CL.16E cells. Several studies have reported results of adherent probiotic strains isolated from different origins which were compared to *L. rhamnosus* (LGG) (recognized probiotic of human origin) (Monteaguedo-Mera *et al*., 2012) and *S. thermophilus* (LMD-9) (Kebouchi *et al*.,

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2016). Our results were in agreement with those revealed by other authors e.g. *Lactococcus lactis* isolated from dairy origin adhered to Caco-2 with percentage of 16% compared to LGG (9.24%) (Monteaguedo-Mera *et al*., 2012). Pisano *et al*. (2014) reported that all lactobacilli strains isolated from Sardanian dairy products were adherent to Caco-2 with percentages ranging from 3 to 20%. In other hand, Guo *et al*. (2012) found that the strains adhered with very important levels to HT29-MTX (66-182%). Adhesive probiotic lactobacilli have been reported to have beneficial health effects, especially dependent on the inhibition of pathogen adhesion to intestinal cell lines (Xu *et al*., 2009). Our strains might well be a good colonizer *in vivo*.

Bacterial adhesion assays to epithelial cell lines are not fully comparable to the complex situation in the human intestinal tract (Federici *et al*., 2014). Nevertheless, examination of adherence of probiotic bacteria is essential for understanding the mechanisms involved in the adhesion process and for obtaining information concerning the species and strain differences.

Finally, we obtained forty-three strains isolated from Tunisian sheep and goat milks. These strains possessed an important resistance to the gastrointestinal conditions and showed desirable surface property. They also adhered efficiently to intestinal epithelial cells Caco-2 TC7, HT29-MTX, and HT29-CL.16E. Thus, they may be classified as potential probiotics and they are needed, further to confirm their probiotic potential *in vivo* for use in nutritional and therapeutic dairy products which were beneficial to human health.

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خواص چسبندگی ریسه های پروبایوتیک لاکتوباسیلوس جدا شده از شیر گوسفند و بز تونسی

ا. محمودی، و. بن موسی، ت. ا. خلدی، م. کبوچی، س. سولیگوت هوگنون، ی. لروکس، و م. حسونا

چکیده

در ابتدای آزمایش، چهارصد ریسه جدا شده از شیر گوسفند و بز تونسی از نظر توانایی بقا در دستگاه گوارش غربال شد. از میان آنها، مقاومت ۴۳ باکتری به پپسین، pH=2، پانکراتین و نمک صفرا در حد ۰٫۳٪ حتی بعد از ۵ ساعت قابل مشاهده بود. شناسایی آنها با استفاده از توالی ژنی 16S rRNAانجام شد و ما به گونه (*Lactobacillus plantarum*) (۲۹ جدایه از شیر گوسفند و ۱۱ جدایه از شیر بز) و *Lactobacillus pentosus*(۲ مورد از شیر گوسفند و ۱ مورد از شیر بز) دست یافتیم. ما توانایی خود-انعقادی و/یا آب گریزی(hydrophobicity) را نشان دادیم. بالاخره، هر دو ریسه M63و C78 سطح مهمی از چسبندگی به سه سلول اپیتلیال دستگاه گوارشی شامل Caco-2 TC7، HT29-MTX، وHT29-CL.16E را بروز دادند. رویهم رفته، بر اساس این خواص، می توان ریسه های لاکتوباسیل را ریسه هایی با فواید امید بخش برای تولید غذا های کاربردی (*functional foods*) برای مصرف کننده قلمداد کرد.

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