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CELL SURFACE PROPERTIES OF HALOTOLERANT PROBIOTIC LACTOBACILLI

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ABSTRACT

Cell surface characteristics of two halotolerant commercial probiotics (Lactobacillus casei Shirota and L. plantarum 299v) and three halotolerant potential probiotics (L. plantarum, L. pentosus and L. acidipiscis) isolated from Chiapas cheese were evaluated as well as the presence of exopolysaccharides (EPS) in their cell surface. Results showed no differences between the commercial probiotics and the strains of potential probiotics analyzed. All lactobacilli had similar autoaggregation levels (between 19.22 to 21.08%) and were able to coaggregate with gram-positive and gram-negative pathogens. The hydrophobicity to non-polar solvents (xylene, octane and hexadecane) was low. L. casei Shirota and L. plantarum isolated from Chiapas cheese had high affinity to chloroform; furthermore, it was possible to demonstrate the presence of capsular exopolysaccharides (CPS) in these strains because of the presence of carboxyl groups. From the results obtained in this work, it may be concluded that low hydrophobicity affinity to chloroform in these strains is related to the presence of CPS and that the higher affinity to chloroform might be related to CPS in the surface of halotolerant lactobacilli.

Indexing terms/Keywords

Halotolerant lactobacilli; Probiotics, Cell Surface Properties; Hydrophobicity, Exopolysaccharides.

Academic Discipline And Sub-Disciplines

Biotecnology, Food Microbiology

SUBJECT CLASSIFICATION

Lactic acid bacteria, probiotics, exopolysaccharides

TYPE (METHOD/APPROACH)

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INTRODUCTION

All interactions of microorganisms with their environment are surface phenomena, and therewith involve the properties of the microbial cell surface that determine the microorganism's ability to adhere to a surface [1]. Bacterial cell surface components (adhesins, polysaccharides and proteins) play major roles in the adherence of lactobacilli to the intestinal epithelium, and these interactions might lead to pathogen exclusion and immunemodulation of host cells [2]. Microorganisms live attached to biotic or abiotic surfaces by forming polymicrobial aggregates called biofilms, generally composed of more than one specie attached to different surfaces and embedded in extracellular polymeric substances produced by the microorganisms [3]. Inside the human body, cells usually adhere to the epithelial mucosa that covers the oral cavity, the gastrointestinal tract (GIT) and the vagina. A good adhesion to the host is required both for pathogenic and probiotic bacteria.

Probiotics are live microorganisms that confer benefits to the host and ameliorate or prevent some diseases [4, 5]. Actually, most of the commercial probiotics belong to *Lactobacillus* and *Bifidobacterium* genera. Lactobacilli contain a great number of species that are able to live in many habitats such as the human gut, vagina and oral cavity [6].

The cell enveope of probiotic bacteria is composed of the lipid bilayer plasma membrane, with embedded proteins, encompassed by the cell wall. The bacterial cell wall consists of a thick multilayered sacculus made of peptidoglycans, along with teichoic acids, exopolysaccharides (EPS), proteinaceous filaments and proteins that are anchored to the cell wall through different mechanisms that are involved in aggregation mechanisms [7, 8]. Some species of lactobacilli display an additional layer of proteins surrounding the peptidoglycan layer called as S-layer [2, 9]. The ability of aggregation may involve the clumping of cells of the same strains (autoaggregation) or different strains (coaggregation). Bacterial probiotic aggregation is related to the ability for both interaction between bacteria close to each other and inhibition of pathogenic adhesion to the intestinal epithelium [10], and it is considered a routine assay for determining the cell adhesion [11]. In the case of probiotic bacteria, autoaggregation provides the release of beneficial substances into the host whilst coaggregation might inhibit or even avoid the adhesion and activity of pathogenic strains. Several studies have been focused on the aggregative properties of potential probiotic microorganisms [12, 13, 14]. As well, the ability to coaggregate might form a barrier that prevents colonization by other pathogenic bacteria [12]. Finally, the physicochemical properties of the bacterial cell surface might affect their adhesion abilities [15]. Finally, the hydrophobicity of the bacterial cell surface s is related with the presence of some component in the cell surface. For example, high hydrophobicity to chloroform is related with the presence of carboxyl groups [16] that are related with the presence of exopolysaccharides (EPS). EPS quantities and/or qualities are expected to play an important role in colonization and survival within the host [8]. Nonetheless, in the last decades their importance has increased because their prebiotic potential. So, the presence of capsular exopolysaccharides (CPS) might be related to the bacterial survival inside the GIT [17] and some healthy benefits have been recently identified, especially their capacity to reduce blood cholesterol [18].

Research about probiotics into salty food matrices has been increasing during the last years [19]. Resistance to stress in probiotic bacteria will be critical in the survival of probiotics from storage to gastric transit and may perform better their health promoting properties [20]. Recently, the halotolerance ability of two commercial probiotics (*Lactobacillus casei Shirota* and *L. plantarum* 299v) was determined [21] along with the probiotic potential of three halotolerant lactobacilli isolated from a Mexican ripened cheese (*Lactobacillus plantarum, L. pentosus* and *L. acidipiscis*) [22].

The aim of the present study was to determine the cell surface properties and the presence of EPS in two halotolerant commercial probiotics (*Lactobacillus casei* Shirota and *L. plantarum* 299v) and three halotolerant potential probiotics isolated from a tropical Mexican salty cheese (Chiapas cheese) (*Lactobacillus plantarum, L. pentosus* and *L. acidipiscis*). For this purpose, the cell surface properties of the five halotolerant lactobacilli were measured as autoaggregation, coaggregation, hydrophobicity and presence of EPS.

MATERIAL AND METHODS

Bacterial strains and growth conditions

Two commercial probiotic strains: *Lactobacillus casei* subs. Shirota (Yakult®) and *Lactobacillus plantarum* subs. 299v (Protransitus LP®) were used to compare the cell surface properties between probiotic and potential probiotic, halotolerant strains *Lactobacillus plantarum, L. pentosus* and *L. acidipiscis* previously isolated, identified and characterized from a tropical salty Mexican cheese [23, 21]. Halotolerance of the five strains was previously confirmed [22]. The five lactobacilli tested were grown in Man Rogosa Sharpe (MRS) broth (Difco, Le Point de Claix, France) at 37ºC under aerobic conditions until their use. Pathogen strains of *Staphylococcus aureus* ATCC 25923, *Salmonella enterica* Servar. *thyphimurium* ATCC 14028, *Listeria monocytogenes* ATCC 19115 and *Escherichia coli* ATCC 43895 were used for the coaggregation assay and grown in Müeller-Hinton broth (Difco, Le Point de Claix, France) at 37 ºC under aerobic conditions until their use. Additionally, *Streptococcus thermophilus* ATCC-BAA 250 was employed as a positive control of EPS production and grown in MRS broth at 37ºC. All the strains were preserved frozen at -20 ºC with glycerol as a cryoprotectant until used.

Aggregation assays

All *Lactobacillu*s strains were grown statically in MRS broth at 37 ºC for 24 h under aerobic conditions, harvested by centrifugation (Hettich Universal RF, Tuttlingen, Germany) (3,000 g, 15 min, 4 ºC) washed twice with Phosphate buffer saline (PBS) (NaCl 8 g/L ; KCl 0.2 g/L; $Na_2HPO_4 \cdot 2H_20$ 1.44 g/L; KH_2PO_4 0.24 g/L; pH 7.2) and resuspended in the same buffer. Absorbance at 600 nm was adjusted to 0.28 ± 0.02 (Jenway 6405 UV/Visible, London, UK).

Autoaggreagation ability was determined as previously described [15]. The bacterial suspension were shaken in vortex (Barnestead / Thermoline Maxi-Mis Plus, Dubuque, Iowa, USA) during 10 s, incubated at 37ºC in a water bath (Polyscience Inc, Niles, Illinois, USA) and monitored during different times (0, 2, 4 h). Autoaggreagation was determinate as follows:

$$Autoaggregagion\ (AA) = \left[\frac{A_{initial} - A_{final}}{A_{initial}}\right] * 100 \quad [1]$$

For coaggregation assay, cell suspensions were prepared as previously described [15]. Equal volumes (4 mL) of each cell suspension (*Lactobacillus* and pathogens strains) were mixed together in pairs by vortexing for 10 s. Control tubes were set up at the same time, containing 8 mL of each bacterial suspension on its own. The absorbance (A) at 600 nm of the suspensions was measured after mixing for 10 s and at different incubation times (2 and 4 h) in a water bath at 37 ºC. Coaggregation was calculated by using two different equations, the Handley et al. 1987 [24] equation:

$$Coaggregation\ (\%) = \left[1 - \frac{A_{mixf}}{\frac{A_1 + A_2}{2}}\right] * 100 \ [2]$$

And the Malik et al. 2003 [25] equation:

$$Coaggregation\ (\%) = \left[1 - \frac{A_{mixi}}{A_{mixf}}\right] * 100 \ [3]$$

Where A_{mixf} is absorbance of the mixture at the end time, A_{mixi} is the absorbance of the mixture at the initial time and A_1 and A_2 are the absorbance at the end time of each strain by separate.

Cell surface hydrophobicity

Cell surface hydrophobicity was determined by the classical Microbial Adhesion to Hydrocarbons (MATH) method developed by [26] modified by [16] with the use of polar solvents. Briefly, all the strains were cultured overnight in MRS broth at 37 ºC. After that, cells were harvested by centrifugation (3,000 g, 15 min, 4 ºC), washed twice with phosphate-urea-magnesium (PUM) buffer ($K_2HPO_4 \cdot 3H_20$ 22.2 g/L; KH_2PO_4:7.26 g/L; Urea 1.8 g/L; $MgSO_4$ 0.2 g/L; pH 7.1) and resuspended in the same buffer to a final absorbance at 560 nm of 0.48 ± 0.08 to standardize the number of bacteria. Non-polar solvents (o-xylene and n-octane) or non-soluble electron acceptor (ethyl acetate) were added to the bacterial suspension in a proportion of 1:4. It is important to comment that in a previous research, with the same five halotolerant lactobacilli, another non-polar solvent was employed with the same strains (n-hexadecane) as well as a non-soluble electron donor (chloroform) [21]. The two-phase system was thoroughly mixed in vortex for 2 min; the aqueous phase was carefully removed after 1 h of incubation in a water bath at 37 ºC and absorbance at 560 nm was determined. Hydrophobicity was measured used the following equation:

$$Hydrophobicity\ (H) = \left[\frac{A_i - A_f}{A_f}\right] * 100 \ [4]$$

Where A_i is the absorbance at 560 nm to the aqueous phase at the beginning of the experiment (without the solvent) and A_f is the absorbance at 560 nm of the aqueous phase at the end of the experiment.

Production of EPS

Congo Red Assay (CRA) was developed as described [27]. Briefly, a Congo red dilution (100 µg/mL H_20) was autoclaved (Tuttnauer 2540E, Brinkmann Instruments, Inc., Westbury, NY, USA) separately and then added to a sterile brain-heart infusion (BHI) supplemented with 36 g/L of sucrose. *Lactobacilli* samples, previously centrifuged (14,000 g, 15 min, 4ºC) and washed twice with PBS buffer, were inoculated into CRA by the drop technique. Then, plates were incubated under aerobic conditions at 37 ºC for 72 h. The presence of colonies was evaluated at 72 h based on their color and morphology by following [28] criteria. *Streptococcus thermophilus* ATCC-BAA 250 was used as a control of non-slime production (intense red colonies).

Ruthenium Red Assay (RRA) was developed by following the methodology proposed by [29]. MRS agar was supplemented with 0.08 g/L ruthenium red. Petri dishes were loop seeded and incubated at 37 ºC for 24 h. EPS-producing colonies were detected by means of the presence of ropy appearance and by touching them with a sterile inoculation loop. Also, the color of the colonies was considered as a factor when ropy colonies appear as white or pink.

Presence of CPS by optical microscopy

The localization of EPS in the cell wall of the *Lactobacillus* strains was carried out by optical microscopy using Toluidine blue and Alcian blue as dyes [30]. Briefly, lactobacilli samples were stained with Toluidine blue (1 g of Toluidine blue, 2 g sodium borate, and distilled water to 100 mL) or Alcian blue (1 g Alcian blue 8GX in 100 mL of 3% (v/v) acetic acid). The presence of CPS was observed by phase contrast microscopy (Nikon, Eclipse 50i, Tokyo, Japan).

Statistical analysis

Results of aggregation and hydrophobicity assays were expressed as the average of three independent experiments done by triplicate in three different days. Each experiment was subjected to one way analysis of variance (ANOVA) followed by

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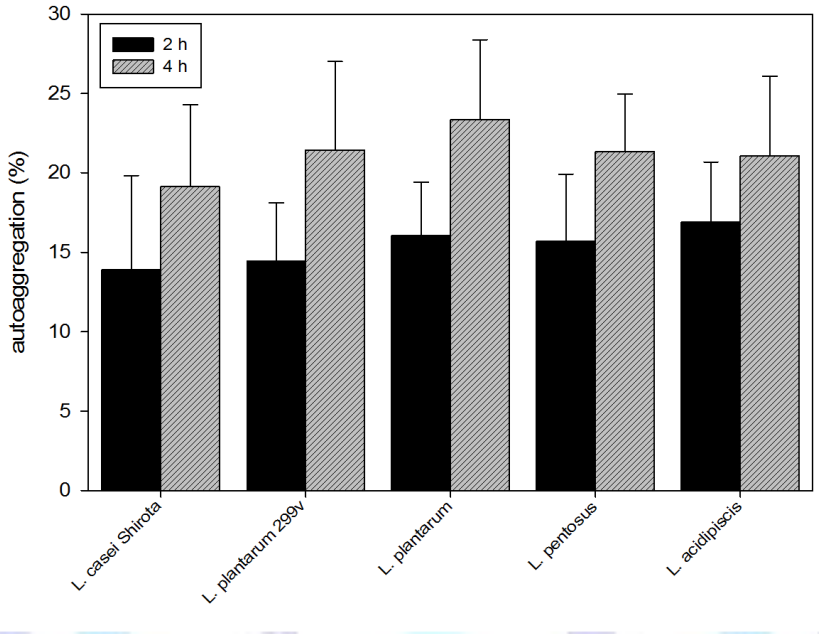
Student-Newman-Keuls test for comparison of means. The level of significance was 2α = 0.05. When convenient, Pearson correlation coefficient was determined with the aim of finding possible relationships between variables. All statistical tests were performed by using MS-Excel® software and Sigma Plot 11.0 (Systat Software Inc., IL, USA).

RESULTS

Aggregation

All strains showed low autoaggregation levels (figure 1) and similar behavior at both 2 and 4 h ($P > 0.05$). The autoaggregation level was significantly higher ($P \leq 0.05$) at 4 h than at 2 h.

Figure 1: Autoaggregation (%) of *Lactobacillus casei* Shirota, *L. plantarum* 299v, *L. plantarum*, *L. pentosus* and *L. acidipiscis* at 37 ºC under aerobic and static conditions at 2 and 4 h.



Coaggregation at 2 and 4 h of each mixture of lactobacilli and pathogens strains were analyzed with equation [2] and equation [3]. Results are shown in table 1.

Coaggregation between halotolerant lactobacilli isolated from Chiapas cheese showed different results using equation [2] and equation [3] (table 2). However, statistically significant differences between these results were not found ($P \geq 0.05$).

Table 1: Coaggregation (%) of *Lactobacillus casei* Shirota, *L. plantarum* 299v, *L. plantarum, L. pentosus* and *L. acidipiscis* to pathogen strains *(Salmonella enterica* Serva. *Thyphimurium* ATCC 14028, *Escherichia coli* ATCC 4389, *Staphylococcus aureus* ATCC 25923 and *Listeria monocytogenes* ATCC 19115) at 2 and 4 h using Handley et al., 1987 equation [2] and equation [3].

Co-aggregation between	[3]			[2]		
	Time (h)		P	Time (h)		P
	2	4		2	4	
E. coli ATCC 4389						
L. plantarum 299v	6.912 ± 3.062	9.049 ± 4.716	0.476	4.850 ± 2.507	8.945 ± 7.795	0.435
L. casei Shirota	4.536 ± 2.455	12.642 ± 3.523	0.003	4.722 ± 0.490	10.386 ± 0.729	≤ 0.001
L. plantarum	5.551 ± 2810	10.585 ± 2.948	0.013	4.329 ± 2.601	8.721 ± 3.811	0.226
L. pentosus	10.976 ± 5.387	15.597 ± 6.651	0.244	4.623 ± 2.968	10.226 ± 6.421	0.164
L. acidipisciss	10.124 ± 6.626	14.996 ± 8.048	0.279	7.451 ± 3.325	9.899 ± 2.033	0.338

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Co-aggregation between	[3]			[2]		
	Time (h)		P	Time (h)		P
	2	4		2	4	
Salmonella 408ntérica servar. *Thyphimurium* ATCC 14028						
L. plantarum 299v	11.244 ± 4.352	13.352 ± 4.173	0.412	8.632 ± 1.901	11.812 ± 3.793	0.184
L. casei Shirota	10.639 ± 3.594	13.913 ± 5.222	0.235	10.619 ± 1.426	12.575 ± 2.927	0.342
L. plantarum	9.378 ± 5.595	15.231 ± 6.418	0.123	8.616 ± 4.702	14.774 ± 6.996	0.231
L. pentosus	9.594 ± 5.047	12.232 ± 4.960	0.407	5.685 ± 2.134	6.938 ± 3.448	0.668
L.acidipiscis	11.007 ± 3.180	14.740 ± 3.052	0.159	11.335 ± 5.079	17.660 ± 2.657	0.054
S. aureus ATCC 25923						
L. plantarum 299v	6.671 ± 1.708	14.173 ± 4.068	0.002	14.826 ± 7.031	22.037 ± 7.704	0.255
L. casei Shirota	7.412 ± 1.633	15.107 v 4.733	0.004	10.097 ± 5.755	19.645 ± 4.706	0.116
L. plantarum	7.412 ± 1.633	11.255 ± 3.248	0.027	13.195 ± 1.138	17.275 ± 9.910	0.620
L. pentosus	9.410 ± 6.158	14.251 ± 4.651	0.155	18.269 ± 5.555	11.262 ± 5.294	0.189
L. acidipiscis	10.124 ± 6.624	9.093 ± 3.485	0.743	15.461 ± 3.657	10.912 ± 5.253	0.259
Listeria monocytogenes ATCC 19115						
L. plantarum 299v	9.746 ± 5.531	13.487 ± 4.487	0.227	5.496 ± 1.895	10.767 ± 3.416	0.036
L. casei Shirota	18.548 ± 3.988	11.626 ± 3.131	0.647	12.003 ± 3.528	12.305 ± 5.948	0.933
L. plantarum	10.010 ± 3.153	12.574 ± 4.730	0.295	12.610 ± 2.319	15.886 ± 2.438	0.080
L. pentosus	8.707 ± 3.675	13.095 ± 3.831	0.070	10.336 ± 4.547	14.221 ± 7.299	0.347
L. acidipiscis	9.529 ± 4.187	11.899 ± 3.171	0.295	11.902 ± 4299	13.850 ± 2.731	0.459

[2] equation proposed by Handley *et al.,* 1987 y [3] equation proposed by Malik *et al*., 2003

Table 2: Coaggregation (%) between (*L. casei Shirota*, *L. plantarum* 299v, *L. plantarum, L. pentosus* and *L. acidipiscis)* at 2 and 4 h using Handley et al., 1987 equation [2] and equation [3] and One-way ANOVA probability (P).

Co-aggregation between	[3]			[2]		
	Time (h)		P	Time (h)		P
	2	4		2	4	
L. plantarum						
L. pentosus	12.978 ± 2.808	20.140 ± 4.711	0.010	17.038 ± 3.212	23.871 ± 6.348	0.103
L. acidipiscis	15.781 ± 2.322	22.354 ± 3.087	0.002	17.333 ± 5.533	23.469 ± 4.440	0.061
L. pentosus						
L. acidipiscis	17.041 ± 3.401	22.334 ± 3.541	0.025	18.408 ± 3.768	23.212 ± 6.749	0.159
Three halotolerant lactobacilli from Chiapas cheese						
	13.642 ± 4.809	19.689 ± 4.612	0.077	15.677 ± 5.820	21.946 ± 4.830	0.087

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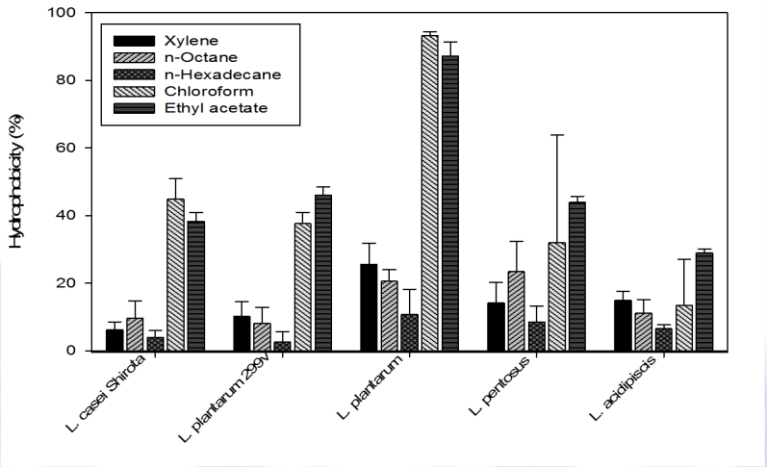
Cell surface hydrophobicity (CSH)

All lactobacilli showed low CSH to the two non-polar solvents (xylene, and octane) (Figure 2). *L. acidipiscis* had the lowest polar hydrophobicity to chloroform and ethylacetate showing a non-polar character in their surface. However *L. casei* Shirota and showed a strong affinity to the previously reported chloroform showing a basic character the presence of carboxyl groups in their cell surface [16].

Figure 2: Hydrophobicity (%) at 560 nn to non-polar solvents (xylene, octane and hexadecane) and to polar solvents (chloroform and ethyl acetate) of *L. casei Shirota, L: plantarum 299v, L. plantarum, L. pentosus and L. acidipiscis*.



Production of EPS and presence of CPS.

The production of EPS and the presence of CPS could not be confirmed in the case of *L. acidipiscis* (table 3). *L. casei* Shirota, *L. plantarum* 299v and *L. plantarum* showed both EPS production and presence of CPS and in the case of *L. pentosus*, presence of CPS could not be confirmed.

Table 3: Production of exopolysaccharides (EPS) using Congo Red Agar assay and Ruthenium Red Agar assay and presence of capsular EPS by stain with Toluidine blue and Alcian blue in *Lactobacillus casei* Shirota, *L. plantarum* 299v, *L. plantarum, L. pentosus* y *L. acidipiscis*.

	EPS production		CPS presence	
Strain	**Congo red agar**	**Ruthenium red agar**	**Toluidine blue**	**Alcian blue**
L. casei Shirota	+	+	+	+
L. plantarum 299v	+	+	+/-	+
L. plantarum	+	+	+	+
L. pentosus	+	+	+	+/-
L. acidipiscis	+/-	+/-	-	+/-

Where + means presence observed, +/- probably present and – presence not observed

DISCUSSION

Aggregation

The aggregation was determined at 2 and 4 h to simulate the normal gastrointestinal transit time with a low ionic strength buffer (PBS). Thus, this assay takes into account all the strength related with the superficial aggregation and there is not possibility to separate the ionic interactions from van der Waals and Lewis acid-base interactions [31].

Results showed similar autoaggregation levels than previously reported for similar strains under similar conditions [32, 33, 34, 35]. But, no references about autoaggregation *of L. acidipiscis* were found. In some cases, a relationship between salt

tolerance of the strains, aggregation abilities and EPS production has been found [36]. However, all the halotolerant strains tested had been showed similar salt tolerance [22].

In the case of coaggregation, equation [2] and equation [3], were used to analyze the phenomenon because both equations are almost equally referred in the database Scopus (59 citations equation [2] *vs.* 57 citations equation [3], as reviewed by April 7, 2014). As found in other research works, the coaggregation index of the lactobacilli was different depending on the equation used [12]. In most cases, coaggregation was similar to previously reported for similar strains [33, 12, 37]. No previous references about *L. acidipiscis* were found. Nevertheless the equation used, in all cases the coaggregation level was low or medium. But, no significant differences ($P > 0.05$) were found between all the mixtures analyzed, despite of the equation employed and the incubation time was not a key factor in most of cases ($P > 0.05$) in spite of the equation used. It is important to indicate that using equation [2] lactobacilli aggregated better with gram-positive pathogens than with gram-negative ones.

The determination of aggregation is important in the formation of biofilms to protect the host from colonization by pathogens [33]. In the present research, all the studied strains formed aggregates that may increase adhesion to epithelial cells. However, differences between results obtained by using both equations make it hard to conclude about the coaggregation ability of the lactobacilli studied. Further research work would be necessary in order to deeply analyze the co-aggregation mechanism and to conclude about the use of one equation that could describe properly this phenomenon.

Cell surface hydrophobicity (CSH)

In a previous research the CSH to n-hexadecane (as non-polar solvent) and chloroform (as electron donor an basic polar solvent) to the same five halotolerant lactobacilli (*Lactobacillus casei* Shirota, *L. plantarum* 299v, *L. plantarum, L. pentosus* and *L. acidipiscis*) was analyzed [21]. However, in order to achieve a better understanding of the complexity of the bacterial cell surface, it was decided to incorporate two other non-polar solvents (xylene and octane) as well as an acidic polar solvent (ethyl acetate). These two solvents have been incorporated to the present discussion (figure 2).

CSH to n-hexadecane showed the lowest hydrophobicity to non-polar solvents in the lactobacilli tested. *L. plantarum, L. pentosus* and *L. acidipiscis* showed higher hydrophobicity to non-polar solvents than *L. casei* Shirota and *L. plantarum* 299v (figure 2). The results obtained with the three non-polar solvents were independent to each other and no Pearson correlation was found between them (*data not shown*) in spite the similar physicochemical properties of the three non-polar solvents analyzed [38] and a great variability in the results were found as in the case of previously reported [39].

Most of the strains showed a high hydrophobicity to both polar solvents and were similar to those previously reported [40, 41]. That suggests a high complexity in the functional groups presents in the cell surface [42, 2]. Moreover, the high affinity to chloroform and the low hydrophobicity to non-polar solvents could be related with the presence of exopolysaccharides in the cell wall [42, 41]. A chloroform / ethyl acetate rate was calculated and a good correlation between chloroform and ethyl acetate was found ($R= 0.961$) indicating a dependence between them.

Microbial adhesion to non-polar solvents reflects the cell surface hydrophobicity, while the values of adhesion to polar solvents have been regarded as a measure of electron donor or acceptor characteristics of bacteria. Consequently, the surface tension of the solvents, the differences between the results obtained with non-polar and polar solvents indicate the occurrence of Lewis acid–base interactions at the bacterial cell surface [15]. Values obtained with the three non-polar solvents and the two polar solvents might be due to similar van der Waals interactions of solvents [38]. For that reason, the difference observed between the organic solvents analyzed and the lactobacilli tested is probably due to the Lewis acid–base interactions resulting from the electron donor-acceptor characteristics of the different bacterial strains.

By comparing the results from the present research with those from previous researches, many differences in experimental conditions were found such as the use of low ionic strength buffers and the presence or absence of oxygen, the initial microbial concentration, the experimental time and temperature and the solvent concentration used. The kind of buffer used is essential because a low-ionic strength buffer (PBS and KNO_3) does not eliminate the hydrophobicity produced by ionic strengths and could generate higher hydrophobicity levels than those obtained with highly ionic buffers [43]. Besides, the presence of oxygen may influence the charges in the cell surface, which increases its hydrophilic character [44].

The higher affinity for chloroform is due to a basic or electron-donating faculty of bacterial cells which might be attributed to the presence of carboxylic groups (usually related to the presence of polysaccharides) on the microbial surface [16] and might be related with the presence of capsular EPS in the cell wall. That could explain the fact that the case that in the strain with the lowest chloroform hydrophobicity level the presence of CPS could not be observed.

Production of EPS and presence of CPS

As previously said, low hydrophobicity values might be related to the presence of EPS in the bacterial cell surface [45]. Moreover, the presence of high hydrophobicity to chloroform could be related with the presence of carboxyl groups in the cell surface that are related with the presence of CPS [16, 46, 47]. So, the low affinity for the chloroform in *L. acidipiscis* and the lack of Alcian blue and Toluidine staining *indicated the short presence of carboxylic groups in their cell wall and so, the absence of CPS.*

CONCLUSIONS

The evidence presented suggests that two halotolerant lactobacilli potential probiotic (*Lactobacillus plantarum, L. pentosus*) had similar physicochemical characteristics in their cell surface than the halotolerant commercial probiotic (*L. casei* Shirota and *L. plantarum* 299v). All the strains were able to coaggregate in a similar way to some gram-positive and gram-negative pathogen strains and showed low hydrophobicity to non-polar solvents. The use of both electron donor and electron acceptor polar solvents contributed to the knowledge about the alkalophilic or acidophilic character of the bacterial surface. The high relation between the low hydrophobicity of the five strains studied and the production of EPS for the halotolerant lactobacilli analyzed support the hypothesis that these two properties are related [48]. Moreover, the presence of CPS seems to be related to the low non-polar hydrophobicity and to the basic character of the cell surface (high affinity for chloroform). However, more work is necessary in order to corroborate this hypothesis.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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