

EVALUATION OF ADHESIVE PROPERTIES OF PRESUMPTIVE PROBIOTIC *Lactobacillus plantarum* STRAINS

AVALIAÇÃO DAS PROPRIEDADES DE ADESÃO DE PRESUMÍVEIS ESTIRPES PROBIÓTICAS DE *Lactobacillus plantarum*

Francesca Silva DIAS¹; Whasley Ferreira DUARTE²; Rosane Freitas SCHWAN³

1. Médica Veterinária, Professora Adjunta do Colegiado de Medicina Veterinária, Universidade Federal do Vale do São Francisco – UNIVASF, Petrolina, PE, Brasil. francesca.nobre@univasf.edu.br; 2. Engenheiro Agrônomo, Professor Adjunto do Departamento de Biologia, Universidade Federal de Lavras – UFLA, Lavras, MG, Brasil; 3. Engenheira Agrônoma, Professora Associada do Departamento de Biologia - UFLA, Lavras, MG, Brasil.

ABSTRACT: Thirty-two strains of *Lactobacillus plantarum* UFLA SAU from pork sausages, pre-selected for some features for probiotic application, were utilized in this study to evaluate their adhesive properties and compare the results against the three pathogens also tested. Strains were tested for autoaggregation and coaggregation capacity and Microbial Adhesion To Solvents (MATS) at the time intervals of 0, 1, 2, 3 and 4 h. Our findings revealed that UFLA SAU strains have a high autoaggregative capacity and coaggregative ability with pathogens, especially *Listeria monocytogenes*. In relation to adhesion to solvents, in general, *L. plantarum* strains showed hydrophilic cell surface properties and an important electron donor and basic character. Adhesive properties were markedly separated for the strains under study by Principal Component Analysis software. UFLA SAU 132, 226 and 87 were differentiated by autoaggregation ability. UFLA SAU 11 and *Listeria monocytogenes* were characterized by adhesion to solvents. UFLA SAU 14, 18 and 172 showed high coaggregation with *Escherichia coli*, *Salmonella* Typhi and *Listeria monocytogenes*. In comparison to the pathogens tested, many UFLA SAU strains presented higher adhesive capacity. These tests should be used for screening and identifying potentially adherent microorganisms. Adhesive properties are important features for the choice of probiotic strains and confer various applications, such as in the pharmaceutical (therapeutic or prophylactic) and food (functional foods) industries.

KEYWORDS: *Lactobacillus plantarum*. Autoaggregation. Coaggregation. Adhesion to solvents.

INTRODUCTION

Lactobacillus plantarum is a member of the facultatively heterofermentative group of lactobacilli. It is a heterogeneous and versatile species that is encountered in a variety of environmental niches, including dairy, meat, fish, and many vegetable or plant fermentations. Moreover, strains of *L. plantarum* have proven ability in surviving gastric transit and colonizing the intestinal tract of humans and other mammals (DE VRIES et al., 2006; GEORGIEVA et al., 2009). The species has been evaluated for its probiotic potential and it is applied as adjunct cultures in various types of food products or in therapeutic preparations. *L. plantarum* strains are used in commercial probiotics in the market characterizing health products (DE VRIES et al., 2006; LEE et al. 2011; JENSEN et al. 2012).

In the screening process for new probiotic strains, there are no clearly established bacterial phenotypic markers that could be used for prediction of the health promotion capacity of lactobacilli (VOLTAN et al., 2007; KOTZAMANIDIS, et al., 2010). However, for the strain to exert a beneficial health effect, its

adherence in the intestine of the host is required. Thus, the adhesion ability to the intestinal epithelium is one of the most important characteristics of lactobacilli, as well as one of the main criteria for selecting probiotic strains (OUWEHAND et al., 1999; CANDELA et al., 2008).

Bacterial adhesion is initially based on non-specific physical interactions between two surfaces, which then enable specific interactions between adhesins (usually proteins) and complementary receptors (PÉREZ et al. 1998; BOS et al. 1999). Autoaggregation of probiotic strains is necessary for adhesion to intestinal epithelial cells, and coaggregation abilities may form a barrier that prevents colonization by pathogenic microorganisms (DEL RE et al. 2000; KOTZAMANIDIS et al., 2010). Physicochemical characteristics of the cell surface, such as hydrophobicity and charges, may affect autoaggregation and adhesion of bacteria to different surfaces (DEL RE et al. 2000; GIAOURIS et al., 2009). The correlation between hydrophobicity and adhesion ability has been observed in some lactobacilli (DEL RE et al. 2000; GIAOURIS et al., 2009; KOTZAMANIDIS et al.,

2010).

The aim of this study was to further investigate the *in vitro* adhesion capacity of 32 presumptive probiotic UFLA SAU *Lactobacillus plantarum* strains isolated from pork sausages by tests of autoaggregation, coaggregation and Microbial Adhesion To Solvents (MATS) and compare the results against the three pathogens also tested.

MATERIAL AND METHODS

Bacterial strains and growth conditions

A total of 32 pre-selected UFLA SAU *Lactobacillus plantarum* among 567 strains from the culture collection of the Department of Biology, Federal University of Lavras, MG, Brazil, were used in this survey. These strains were isolated from pork sausages and they possess some features as criteria for application probiotic: no hemolytic, absence of decarboxylase activity, exopolysaccharide production, antibacterial activity and tolerate the effect of low pH, bile, pancreatic fluid (DIAS et al., 2013).

Standard pathogens strains of *Escherichia coli* (ATCC 8739), *Salmonella* Typhi (ATCC 6539) and *Listeria monocytogenes* (ATCC 7644) were used in this study. All *L. plantarum* were stored at -70 °C in the Man Rogosa Sharpe (MRS) broth (Difco, Detroit, MI, USA) with 30% glycerol. *E. coli* and *S. Typhi* were maintained on nutrient agar (Difco) slopes at 4 °C and *L. monocytogenes* in tryptic soy agar with 0.6% yeast extract.

Autoaggregation assays

Autoaggregation assays were performed as previously described by Kos et al. (2003), with minor modifications. Briefly, the cells were washed twice with phosphate buffered saline (PBS) (pH 7.2). The cells were then resuspended in 4 ml of PBS to 10⁸ CFU/ml by vortexing for 10 s and incubated for 4 h at room temperature. At times 0, 1, 2, 3 and 4 h, 5 µl of the upper suspension was carefully removed, transferred to microplate containing 195 µl of PBS, and the absorbance (A) at 620 nm was measured. The autoaggregation percentage was expressed as a function of time until it was constant, using the formula: $1 - (A_t/A_0) \times 100$, where A_t represents the absorbance at time t= 1, 2, 3 for 4 h and A₀ the absorbance at t=0.

Coaggregation assays of pathogens with *L. plantarum* strains

The method for preparing the cell suspensions used for testing coaggregation was the

same as the autoaggregation assay as suggested by Kos et al. (2003). Equal volumes (2 ml) of each *Lactobacillus* and pathogenic strain were mixed by vortexing for 10 s. Control tubes were set up at the same time, containing 4 ml of each separate bacterial suspension. The A at 620 nm of the suspensions was measured after mixing and after 4 h of incubation at room temperature. Samples were taken in the same way as in the autoaggregation assay. The percentage of coaggregation was calculated using the equation of Handley et al. (1987):

$$\text{Coaggregation (\%)} = \frac{((A_{\text{Lactob}} + A_{\text{pathog}})/2) - A_{\text{mix}}}{A_{\text{Lactob}} + A_{\text{pathog}}} \times 100,$$

where A_{pathog} and A_{Lactob} represent the A₆₂₀ nm of the separate bacterial suspensions, and A_{mix} represents the absorbance of the mixed bacterial suspension.

Microbial Adhesion To Solvents (MATS) measurement

MATS was measured according to the method proposed by Pelletier et al. (1997) with modifications. In this study, three solvents (Merck) were tested for adherence to *Lactobacillus* and pathogenic strains: xylene (apolar solvent), chloroform (monopolar and Lewis-acid solvent) and ethyl acetate (monopolar and Lewis-base solvent). The microbial adhesion to xylene, chloroform and ethyl acetate reflect cell surface hydrophobicity as well as the electron donor/basic and electron acceptor/acidic characteristics of bacteria, respectively.

Stationary phase cells were washed twice in PBS and resuspended in 3 ml of 0.1 M KNO₃ to a final concentration of approximately 10⁸ CFU/ml bacteria (cell suspension). One milliliter of each solvent was then added to the cell suspension to form a two-phase system. After a 10 min pre-incubation at room temperature, the two-phase system was mixed by vortexing for 2 min and incubated for 30 min at room temperature to allow phase separation. The aqueous phase (A_t) was carefully removed (200 µl) and added to a microplate (96 wells - Denmark®). The cell suspension (A₀) (200 µl) was also added to a microplate. The absorbance at 620 nm of each sample was measured (Multiskan FC-ThermoScientific Uniscience), and the percentage of cell surface hydrophobicity (H%) was calculated using the formula: $H\% = (1 - A_t/A_0) \times 100$.

Statistical analysis

All tests were performed in triplicate. For coaggregation and MATS, the data were analyzed

using ANOVA, and the means were compared by a Scott-Knott test. A randomized complete design was used for the autoaggregation, coaggregation and MATS methods. To autoaggregation assays, the treatments were arranged in a factorial 35 X 4 design: 35 strains and 4 time points (1, 2, 3 and 4 h). For coaggregation, in time of 4 h, the treatments were arranged in the factorial 32 X 3: 32 UFLA SAU *L. plantarum* strains and three pathogenic microorganisms. For the MATS test, in time of 4 h the factorial was 35 X 3: 35 strains and three solvents. Quantitative data were analyzed using regression. The statistical analysis was performed using SISVAR® (Lavras, Brazil) software, version 4.5.

All *Lactobacillus* properties were analyzed by Principal Component Analysis (PCA) using the software XLSTAT 7.5.2 (Addinsoft, New York, NY, USA).

RESULTS AND DISCUSSION

Autoaggregation assays

The UFL SAU *Lactobacillus* strains were examined for their autoaggregation ability (Table 1). Aggregation is a phenotype related to cell adherence properties (PELLETIER et al., 1997; KOS et al., 2003). Our strains showed a strong autoaggregating phenotype. According to Del Re et al. (2000), strains with values lower than 10% are designed as non-autoaggregating. Thus, in this study, at the time interval of 1 h, a total of 10 *L. plantarum* strains and all three pathogens presented value below of 10%, however, at the time interval of 2 h, all strains surpassed this percentage.

There was an interaction ($P < 0.05$) between the *L. plantarum* strains and evaluation time. Twenty three *L. plantarum* strains, as well as the pathogens, increased linearly over time, as can be explained by the first-degree equations in Table 1. Through the second-degree equation, the UFLA SAU strains 125, 127, 130, 135, 172, 185, 186, 213 and 258 showed higher autoaggregation capacity between 2.9 to 3.5 h and from these time points (specific for each strain), there is a decreasing quadratic of percentage autoaggregative in function of time of study.

Compared to the autoaggregation capacity of pathogens, at the time of 4 h, 31 and 18 *Lactobacillus* strains were more efficient than *E. coli* and *S. Typhi*, respectively. In general, probiotic strains should show higher autoaggregation capabilities than pathogenic strains (COLLADO et al., 2007).

The UFLA SAU 52 strain was the only strain to show a greater capacity to autoaggregate than *L. monocytogenes*. This result indicates that the UFLA SAU 52 possess high potential ability to adhere to epithelial cells and mucosal surfaces. The ability to adhere to epithelial cells and mucosal surfaces has been suggested as an important property of many bacterial strains used as probiotics (BAO et al., 2010; KOTZAMANIDIS et al., 2010). Several studies have investigated the composition, structure and forces of interaction related to bacterial adhesion to intestinal epithelial cells (PELLETIER et al., 1997; PÉREZ et al., 1998; DEL RE et al., 2000; BAO et al., 2010; KOTZAMANIDIS et al., 2010).

Table 1. Autoaggregation percentage of 32 UFLA SAU strains of *L. plantarum* and three pathogens microorganisms.

Strains	Time (h)				Average	Equation	R ² (%)
	1	2	3	4			
1	16.96 ^c	29.34 ^f	29.43 ^d	50.08 ^h	31.45 ^g	9.946 x + 6.587	87.42
11	15.36 ^e	32.54 ^g	33.68 ^e	43.32 ^f	31.22 ^g	8.501 x + 9.971	89.09
14	6.92 ^b	22.46 ^c	30.41 ^d	41.76 ^e	25.39 ^c	11.248 x - 2.731	98.38
18	10.71 ^c	27.33 ^e	32.57 ^e	41.21 ^e	27.96 ^e	9.674 x + 3.772	94.57
20	10.44 ^c	33.07 ^g	41.66 ^g	42.57 ^f	31.94 ^g	10.498 x + 5.690	82.13
34	7.50 ^b	23.59 ^c	36.41 ^f	39.04 ^d	26.64 ^d	10.745 x - 0.228	92.37
52	48.81 ⁱ	60.19 ^j	62.35 ^j	77.20 ^l	62.14 ⁿ	8.733 x + 40.303	93.38
73	14.44 ^c	28.46 ^e	30.25 ^d	34.98 ^c	27.04 ^d	6.338 x + 11.188	85.85
86	8.65 ^b	33.68 ^g	53.16 ⁱ	57.35 ^j	38.21 ^k	16.556 x - 3.181	92.36
87	13.45 ^d	23.41 ^c	47.47 ^h	58.37 ^j	35.67 ⁱ	15.884 x - 4.035	97.13
91	9.51 ^b	33.66 ^g	39.61 ^g	42.85 ^f	31.41 ^g	10.595 x + 4.922	82.22
101	29.53 ^h	43.78 ⁱ	48.44 ^h	57.82 ^j	44.89 ^m	8.950 x + 22.513	96.13
125	7.36 ^b	26.66 ^d	26.90 ^c	26.98 ^b	21.98 ^b	- 4.803 x ² + 29.928 x - 16.821	93.74
127	8.51 ^b	35.47 ^h	37.22 ^f	38.62 ^d	29.96 ^f	- 6.388 x ² + 41.148 x - 25.003	94.99

130	8.74 ^b	30.73 ^f	31.76 ^e	33.03 ^c	26.07 ^d	- 5.179 x ² + 33.283 x - 18.300	94.42
131	13.59 ^d	30.50 ^f	36.12 ^f	39.18 ^d	29.85 ^f	8.241 x + 9.243	86.77
132	26.23 ^g	38.40 ^h	39.03 ^f	58.81 ^j	40.62 ^l	9.838 x + 16.020	88.72
135	16.27 ^e	30.19 ^f	47.18 ^h	42.26 ^f	33.97 ^h	- 4.71 x ² + 33.046 x -13.316	94.53
145	28.73 ^h	46.29 ^k	48.33 ^h	53.55 ⁱ	44.23 ^m	7.651 x + 25.096	84.03
172	11.82 ^c	37.33 ^h	41.49 ^g	40.92 ^e	32.89 ^h	-6.517 x ² + 41.736 x -22.569	97.71
185	7.29 ^b	24.41 ^c	30.58 ^d	22.37 ^a	21.16 ^b	- 6.333 x ² + 36.81 x -23.362	99.80
186	13.64 ^d	35.71 ^h	37.35 ^f	36.32 ^d	30.75 ^g	- 5.776 x ² + 35.849 x - 15.55	95.98
187	5.63 ^b	10.44 ^a	26.47 ^c	40.67 ^e	20.8 ^b	12.115 x - 9.486	95.99
204	20.54 ^f	24.09 ^c	46.77 ^h	55.47 ^j	36.72 ^j	12.745 x + 4.855	92.97
213	15.37 ^e	41.48 ⁱ	48.46 ^h	49.02 ^h	38.58 ^k	- 6.388 x ² + 42.735 x - 20.344	98.93
217	10.80 ^c	27.47 ^e	48.55 ^h	45.63 ^g	33.11 ^h	12.557 x + 1.718	85.26
220	11.72 ^c	33.53 ^g	48.46 ^h	46.88 ^g	35.15 ⁱ	12.042 x + 5.041	83.67
226	13.37 ^d	24.66 ^c	33.55 ^e	53.27 ⁱ	31.21 ^g	12.86 x - 0.937	96.89
238	12.72 ^d	29.79 ^f	42.52 ^g	45.58 ^g	32.65 ^h	11.129 x + 4.828	92.46
245	6.62 ^b	26.52 ^d	30.06 ^d	34.24 ^c	24.36 ^c	8.642 x + 2.753	83.05
258	11.48 ^c	36.36 ^h	40.15 ^g	39.99 ^e	32.00 ^g	- 6.26 x ² + 40.235 x - 21.637	97.43
265	10.84 ^c	26.17 ^d	38.17 ^f	38.53 ^d	38.43 ^e	9.508 x + 4.655	88.38
<i>E. coli</i>	1.18 ^a	16.30 ^b	17.20 ^a	28.70 ^b	15.84 ^a	8.347 x - 5.023	91.08
<i>S. Typhi</i>	2.71 ^a	14.80 ^b	21.38 ^b	41.50 ^e	20.10 ^b	12.292 x -10.638	95.66
<i>Listeria</i>	1.99 ^a	15.64 ^b	47.74 ^h	62.24 ^k	31.90 ^g	21.284 x -21.307	97.20

For each column, mean values with different letters are significant ($P < 0.005$) according to the Scott-Knott test.

^lStandard Error (SE): 0.955

Coaggregation assays of pathogens with *L. plantarum* strains

Coaggregation of UFLA SAU *L. plantarum* strains with three enteropathogens were also examined. In the time of evaluation of this

study, the ability of coaggregation with the pathogens was significantly ($P < 0.05$) greater in the time of 4 h (Table 2). According to Bao et al. (2010), coaggregation property is strain-specific and the degree gradually increases over time.

Table 2. Average percentage of coaggregation activity and Microbial Adhesion To Solvents (MATS) of 32 UFLA SAU *L. plantarum* strains over time from 1 to 4h.

	Time (h)	<i>E. coli</i> ¹	<i>S. Typhi</i> ²	<i>L. monocytogenes</i> ³
Coaggregation (%)	1	11.55 ^a	12.66 ^a	21.11 ^a
	2	17.69 ^b	16.66 ^b	23.56 ^b
	3	22.58 ^c	20.58 ^c	30.16 ^c
	4	27.71 ^d	25.07 ^d	34.70 ^d
	Time (h)	Xylene ⁴	Ethyl acetate ⁵	Chloroform ⁶
MATS (%)	1	32.33 ^a	15.35 ^a	26.29 ^a
	2	32.87 ^b	22.31 ^b	39.19 ^b
	3	33.45 ^c	30.67 ^c	51.33 ^c
	4	33.87 ^c	36.49 ^d	63.53 ^d

For each column, mean values with different letters are significant ($P < 0.005$) according to the Scott-Knott test.

¹SE: 0.394; ²SE: 0.315; ³SE: 0.439; ⁴SE: 0.157; ⁵SE: 0.331; ⁶SE: 0.395.

All of the strains coaggregated with the pathogens except strain UFLA SAU 132, which did not show any coaggregation with the pathogens tested (Figure 1). The coaggregation abilities of the *Lactobacillus* species with potential pathogens might prevent the colonization of the gut by pathogenic bacteria and constitute an important host defense mechanism against infection in the urogenital and gastrointestinal tract. Coaggregation with potentially gut pathogens could therefore

contribute to the probiotic properties ascribed to lactic acid bacteria. (KOS et al., 2003). Thus, probiotic strains should show the ability to coaggregate with the pathogenic strains tested, but the percentage of coaggregation is strain-specific (COLLADO et al., 2007).

In our study, strains of *Lactobacillus* UFLA SAU 185, 91 and 52 showed greater coaggregation with *E. coli*, *S. Typhi* and *L. monocytogenes*, respectively. In relation to the

pathogenic strains tested, at the time interval of 4 h, the UFLA SAU strains showed the highest average coaggregation ($P < 0.005$) with *Listeria monocytogenes*. This property may be related to

the formation of a mixed species biofilm since mixed species biofilms of *L. monocytogenes* and *L. plantarum* have been reported by Veen and Abee (2011).

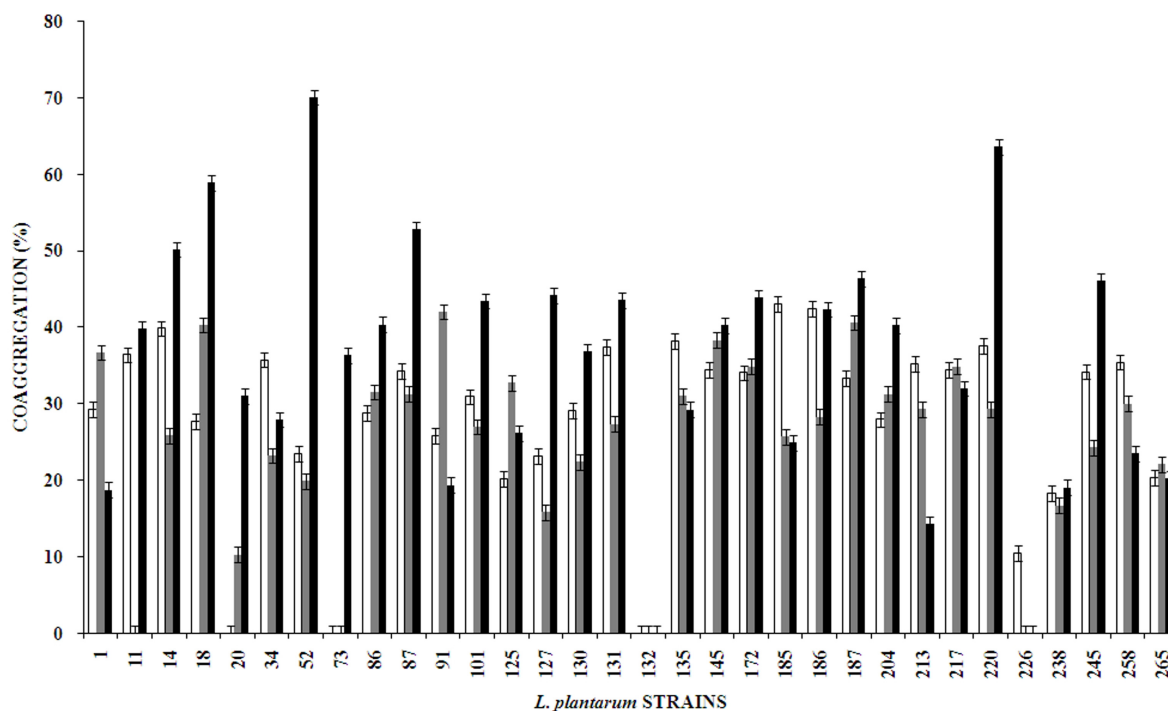


Figure 1. Percent (%) coaggregation of 32 UFLA SAU *L. plantarum* strains to three pathogens: *E. coli* (□), *S. Typhi* (■) and *L. monocytogenes* (■) at the time of 4 h.

Microbial Adhesion To Solvents (MATS) measurement

The MATS method was used to evaluate the hydrophobic/ hydrophilic cell surface properties of *L. plantarum* strains and compare them with the cell surface properties of *E. coli*, *S. Typhi* and *L. monocytogenes*. At the time intervals evaluated in this study, the highest adhesion to solvents took place after 4 h, for the 32 *L. plantarum* strains evaluated. (Table 2).

The results indicated that UFLA SAU 11 and 132 were more hydrophobic, with strong adhesion to xylene, whereas other strains showed lower percentages of adherence to this apolar solvent, such as UFLA SAU 14, 18 and 91 (Figure 2). *L. plantarum* showing an affinity to an apolar solvent above 40% generally present elevated hydrophobic characteristics (GIAROUS et al., 2009). In this study, five *L. plantarum* strains presented a hydrophobic surface (UFLA 11, 125, 132, 220 and 258) with affinity above 40% to xylene. Cell surface hydrophobicity methods do not measure the intrinsic microbial cell surface hydrophobicity, but rather the bacterial adhesion to a certain hydrophobic substratum (KOS et al.

2003). According to Del Re et al. (2000) and Giarous et al. (2009), strains should present a hydrophobic surface for a high capacity of adhesion to intestinal cells and solid materials.

There was an interaction ($P < 0.05$) between the strains and solvents tested at the time of 4 h. In relation to the solvents tested, the UFLA SAU strains showed the highest average to affinity to chloroform. Twenty-nine strains of *Lactobacillus*, as well as the pathogenic strains tested, showed a strong overall affinity to chloroform, an acidic solvent and electron acceptor. UFLA SAU 14, 18 and 172 presented a higher affinity for ethyl acetate, a basic solvent (Figure 2). These results indicate that the metabolic set of enzymes is better electron donor and at the same time weak electron acceptors, as confirmed by their hydrophilic cell surface properties. In other words, lactobacilli have a strong basic and a weak acidic character. According to Pelletier et al. (1997), the quantitatively important existence of chemical groups such as $-\text{COO}^-$ and $-\text{HSO}_3^-$ at the surface of microorganisms could explain their strong electron donor character.

In the MATS test, almost all of the strains

were electron donors because their affinity to the Lewis-acid chloroform was higher than that to the apolar solvent. Moreover, two strongly Lewis-base strains (UFLA SAU 1 and 187) were also identified (more than 50% of difference between affinity to Lewis-acid chloroform and apolar xylene) which indicates the specific potential of those strains to react with polar substratum. These results were similar to those reported by Giaouris et al. (2009) who analyzed *Lactobacillus lactis* strains isolated from animal and vegetables.

In this study, the percentage of adhesion of

pathogens to solvents was tested for comparison with *L. plantarum* strains (Figure 2). Compared to lactobacilli, *L. monocytogenes* showed a higher ability to adhere to xylene, an apolar solvent (64.61%); this high percentage of adhesion to xylene can be justified because the bacteria possess the ability to form biofilms. Adhesion, facilitated by bacterial cell surface hydrophobicity, is defined as the first phase of biofilm formation (TRESSE et al., 2006). *E. coli* and *S. Typhi* showed percentages of adherence to xylene that were slightly higher than the average of the UFLA SAU strains.

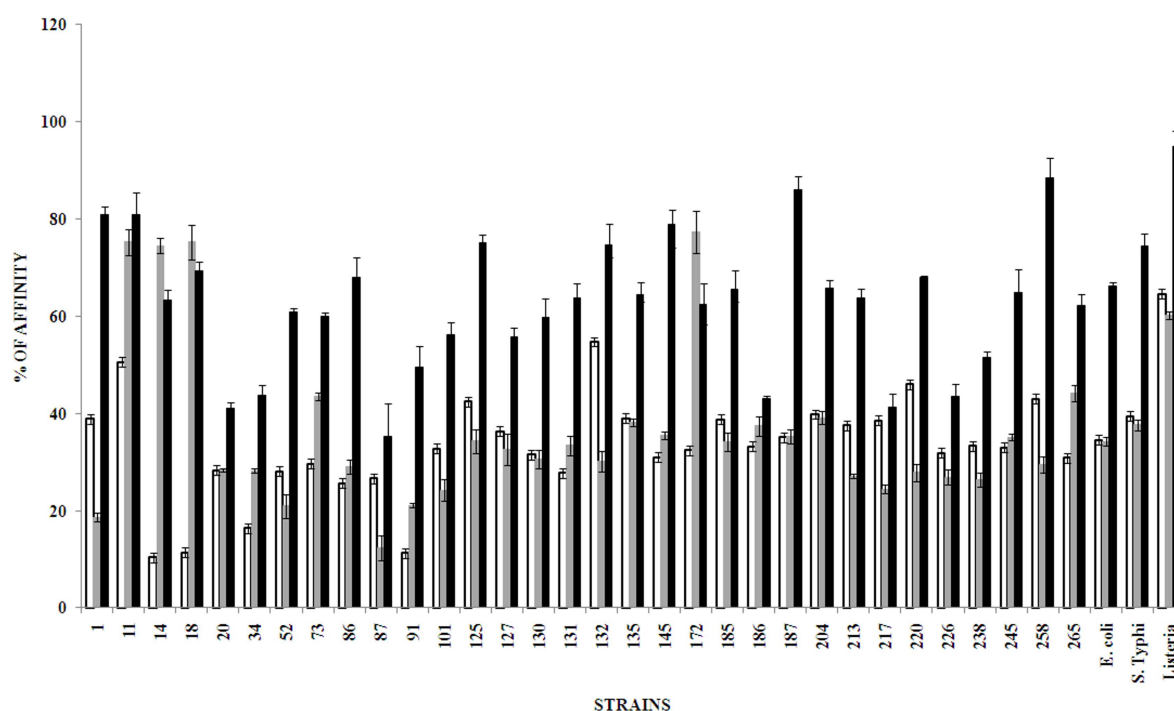


Figure 2. Percent (%) of adhesion of 32 UFLA SAU *L. plantarum* strains, *E. coli*, *S. Typhi* and *L. monocytogenes* to solvents: xylene (□), ethyl acetate (■) and chloroform (■) at the time of 4 h.

Adhesive properties distinction of UFLA SAU strains by PCA

To discriminate the adhesive properties distinction of UFLA SAU strains, PCA was carried out based on their adhesion to solvents as well as their auto and coaggregative capacity. The PCA results presented in Figure 3, show that two components, which account for 50.61% of the variability of the original data set have been extracted, and, PC1 and PC2 explained 32.27% and 18.34% of the total variance, respectively. Adhesive properties were markedly separated in the plane of the biplot. In the lower quadrant of the plane, UFLA SAU 132, 226 and 87 could be differentiated by autoaggregation. In the upper left quadrant of the plane, the strain UFLA SAU 11 and the pathogen microorganism *L. monocytogenes*

were characterized by adhesion to solvents. In the upper right quadrant, UFLA SAU 14, 18 and 172 could be differentiated by coaggregation with *E. coli*, *S. Typhi* and *L. monocytogenes*.

Although, in general, the *L. plantarum* strains have good autoaggregative, and coaggregative ability and adhesion to solvents, the analysis of each particular strain is advantageous because natural diversity of the species occurs. According to Izquierdo et al. (2009) and Jensen et al. (2012), the process of adhesion appears to be multifactorial as adhesion cannot be attributed to one component and includes electrostatic interactions, hydrophobic interactions, and specific bacterial structures. Thus, the screening and distinction of *L. plantarum* strains for the desired properties should be conducted for better results.

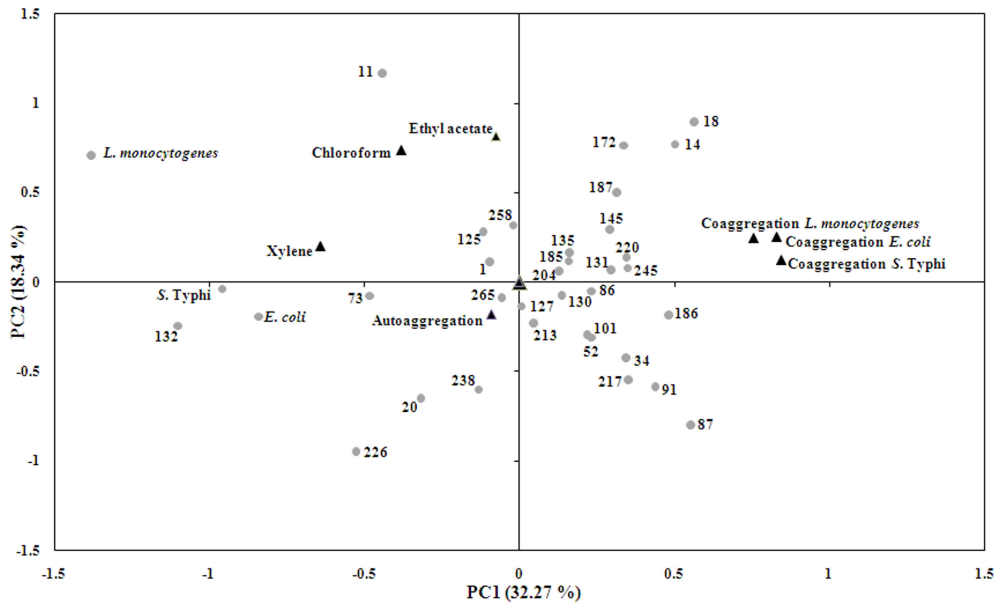


Figure 3. Principal component analysis (PCA) based in adhesion properties data of the 32 UFLA SAU *L. plantarum* strains. The first seven components explained 50.61% of the total variance; among them, PC1 and PC2 explained 32.27% and 18.34% of the total variance, respectively.

CONCLUSIONS

Our findings revealed that UFLA SAU strains have a high autoaggregation and coaggregative ability with pathogens, especially *Listeria monocytogenes*.

In relation to the adhesion to solvents, in general, the *L. plantarum* strains showed hydrophilic cell surface properties and an important electron donor and basic character.

In comparison to the pathogens tested, many strains UFLA SAU presented higher adhesive capacity. The described natural diversity of the autoaggregation, coaggregation and adhesion to solvents of *L. plantarum* affords an important

pool of functionalities for industrial and safety exploitations such as biofilm formation to solid surfaces and commercial probiotic inoculants for therapeutic or prophylactic preparations and functional foods.

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RESUMO: Trinta e duas estirpes de lingüiça suína, *Lactobacillus plantarum* UFLA SAU, pré-selecionadas com algumas características para aplicação probiótica, foram utilizadas neste estudo para avaliar suas propriedades adesivas e comparar os resultados com três patógenos também testados. As estirpes foram testadas para autoagregação, coagregação e capacidade de adesão microbiana aos solventes (MATS) nos tempos de 0, 1, 2, 3 e 4 h. Nossos resultados revelaram que estirpes UFLA SAU apresentam alta capacidade autoagregativa e coagregativa com patógenos, especialmente com *Listeria monocytogenes*. Em relação à adesão aos solventes, de um modo geral, as estirpes de *L. plantarum* mostraram propriedades hidrofílicas de superfície celular e um importante caráter básico e elétron doador. Propriedades adesivas foram marcadamente separadas para as estirpes em estudo através da Análise de Componentes Principais. UFLA SAU 132, 226 e 87 foram diferenciadas pela capacidade de autoagregação. UFLA SAU 11 e *Listeria monocytogenes* foram caracterizadas por adesão aos solventes. UFLA SAU 14, 18 e 172 apresentaram coagregação com *Escherichia coli*, *Salmonella Typhi* e *Listeria monocytogenes*. Em comparação aos patógenos testados, muitas estirpes UFLA SAU apresentaram maior capacidade adesiva. Estes testes podem ser úteis para a triagem e identificação de micro-organismos potencialmente aderentes. Propriedades adesivas são importantes características para a escolha de estirpes probióticas e conferem várias aplicações, tais como nas indústrias: farmacêutica (terapêutico ou profilático) e de alimentos (alimentos funcionais).

PALAVRAS-CHAVE: *Lactobacillus plantarum*. Autoagregação. Coagregação. Adesão aos solventes.

REFERENCES

- BAO, Y.; ZHANG, Y.; ZHANG, Y.; LIU, Y.; WANG, S.; DONG, X.; WANG, Y.; ZHANG, H. Screening of potential probiotic properties of *Lactobacillus fermentum* isolated from traditional dairy products. **Food Control**, Oxford, v. 21, n.5, p. 695-701, 2010.
- BOS, R.; VAN DER MEI, H. C.; BUSSCHER, H. J. Physico-chemistry of initial adhesive interactions—its mechanisms and methods for study. **FEMS Microbiology Reviews**, Amsterdam, v. 23, n. 2, p. 179–230, 1999.
- CANDELA, M.; PERNA, F.; CARNEVALI, P.; VITALI, B.; CIATI, R.; GIONCHETTI, P.; RIZELLO, F.; CAMPIERI, M.; BRIGIDI, P. Interaction of probiotic *Lactobacillus* and *Bifidobacterium* strains with human intestinal epithelial cells: Adhesion properties, competition against enteropathogens and modulation of IL-8 production. **International Journal of Food Microbiology**, Amsterdam, v. 125, n. 3, p. 286–292, 2008.
- COLLADO, M. C.; MERILUOTO, J.; SALMINEN, S. Measurement of aggregation properties between probiotics and pathogens: *In vitro* evaluation of different methods. **Journal of Microbiological Methods**, Amsterdam, v. 71, n. 1, p. 71–74, 2007.
- DEL RE, B.; SGORBATI, B.; MIGLIOLI, M.; PALENZONA, D. Adhesion, autoaggregation and hydrophobicity of 13 strains of *Bifidobacterium longum*. **Letters in Applied Microbiology**, Oxford, v. 31, n. 6, p. 438–442, 2000.
- DE VRIES, M. C.; VAUGHAN, E. E.; KLEEREBEZEM, M.; DE VOS, W. M. *Lactobacillus plantarum* - survival, functional and potential probiotic properties in the human gastrointestinal tract. **International Dairy Journal**, Oxford, v. 16, n. 9, p. 1018-1028, 2006.
- DIAS, F. S.; DUARTE, W. F.; SANTOS, M. R. R. M.; RAMOS, E. M.; SCHWAN, R. F. Screening of *Lactobacillus* isolated from pork sausages for potential probiotic use and evaluation of the microbiological safety of fermented products. **Journal of Food Protection**, Des Moines, v. 76, n. 6, p. 991-998, 2013.
- GEORGIEVA, R.; ILIEV, I.; HAERTLÉ, T.; CHOBERT, J.M.; IVANOVA, I.; DANOVA, S. Technological properties of candidate probiotic *Lactobacillus plantarum* strains. **International Dairy Journal**, Oxford, v. 19, n. 11, p. 696-702, 2009.
- GIAOURIS, E.; CHAPOT-CHARTIER, M. P.; BRIANDET, R. Surface physicochemical analysis of natural *Lactococcus lactis* strains reveals the existence of hydrophobic and low charged strains with altered adhesive properties. **International Journal of Food Microbiology**, Amsterdam, v. 131, n. 1, p. 2–9, 2009.
- HANDLEY, P. S.; HARTY, D. W. S.; WYATT, J. E.; BROWN, C. R.; DORAN, J. P.; GIBBS, A. C. C. A comparison of the adhesion, coaggregation and cell-surface hydrophobicity properties of fibrillar and fimbriate strains of *Streptococcus salivarius*. **Journal of General Microbiology**, London, v. 133, n. 11, p. 3207–3217, 1987.
- IZQUIERDO, E.; HORVATOVICH, P.; MARCHIONI, E.; AOUDE-WERNER, D.; SANZ, Y.; ENNAHAR, S. 2-DE and MS analysis of key proteins in the adhesion of *Lactobacillus plantarum*, a first step toward early selection of probiotics based on bacterial biomarkers. **Electrophoresis**, Weinheim, v. 30, n. 6, p. 949–956, 2009.
- KOS, B.; SUSKOVIC, J.; VUKOVIC, S.; SIMPRAGA, M.; FRECE, J.; MATOSIC, S. Adhesion and aggregation ability of probiotic strain *Lactobacillus acidophilus* M92. **Journal of Applied Microbiology**, Oxford, v. 94, n. 6, p. 981–987, 2003.
- KOTZAMANIDIS, C.; KOURELIS, A.; LITOPOULOU-TZANETAKI, E.; TZANETAKIS, N.; YIANGOU, M. Evaluation of adhesion capacity, cell surface traits and immunomodulatory activity of presumptive probiotic *Lactobacillus* strains. **International Journal of Food Microbiology**, Amsterdam, v. 140, n. 2-3, p. 154-163, 2010.

- LEE, J.; YUN, H. S.; CHO, K. W.; OH, S.; KIM, S. H.; CHUN, T.; KIM, B.; WHANG, K. Y. Evaluation of probiotic characteristics of newly isolated *Lactobacillus* spp.: Immune modulation and longevity. **International Journal of Food Microbiology**, Amsterdam, v. 148, n. 2, p. 80–86, 2011.
- JENSEN, H.; GRIMMER, S.; NATERSTAD, K.; AXELSSON, L. *In vitro* testing of commercial and potential probiotic lactic acid bacteria. **International Journal of Food Microbiology**, Amsterdam, v. 153, n. 1-2, p. 216–222, 2012.
- OUWEHAND, A. C.; KIRJAVAINEN, P. V.; SHORTT, C.; SALMINEN, S. Probiotics: mechanisms and established effects. **International Dairy Journal**, Oxford, v. 9, n. 1, p. 43–52, 1999.
- PELLETIER, C.; BOULEY, C. C.; BOUTTIER, S.; BOURLIOUX, P.; BELLON-FONTAINE, M. N. Cell surface characteristics of *Lactobacillus casei* subsp. *casei*, *Lactobacillus paracasei* subsp. *paracasei*, and *Lactobacillus rhamnosus* strains. **Applied Environmental Microbiology**, Washington, v. 63, n. 5, p. 1725–1731, 1997.
- PÉREZ, P.; MINAARD, Y.; DISALVO, E.; DE ANTONI, G. Surface properties of bifidobacterial strains of human origin. **Applied Environmental Microbiology**, Washington, v. 64, n. 1, p. 21–26, 1998.
- TRESSE, O.; LEBRET, V.; BENEZECH, T.; FAILLE, C. Comparative evaluation of adhesion, surface properties, and surface protein composition of *Listeria monocytogenes* strains after cultivation at constant pH of 5 and 7. **Journal of Applied Microbiology**, Oxford, v. 101, n. 1, p. 53–62, 2006.
- VEEN, S. V. D.; ABEE, T. Mixed species biofilms of *Listeria monocytogenes* and *Lactobacillus plantarum* show enhanced resistance to benzalkonium chloride and peracetic acid. **International Journal of Food Microbiology**, Amsterdam, v. 144, n. 3, p. 421–431, 2011.
- VOLTAN, S.; CASTAGLIUOLO, I.; ELLI, M.; LONGO, S.; BRUN, P.; D'INCA, R.; PORZIONATO, A.; MACCHI, V.; PALU, G.; STURNIOLO, G. C.; MORELLI, L.; MARTINES, D. Aggregating phenotype in *Lactobacillus crispatus* determines intestinal colonization and TLR2 and TLR4 modulation in murine colonic mucosa. **Clinical and Vaccine Immunology**, Washington, v. 14, n. 9, p. 1138–1148, 2007.