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In vitro probiotic potential of *Lactobacillus* spp. strains from the vagina of dairy cows

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Abstract

The objective of the study was to evaluate the *in vitro* probiotic potential of 28 *Lactobacillus* spp. strains, isolated from the vagina of creole dairy cows of the Manabí region –Ecuador–, for its utilization as probiotic in cows with urogenital disorders. The pH decrease and growth capacity at 24 h was determined, and the survival to pH (6, 8 and 9) and temperature (38, 39 and 40 ºC), the adherence to hydrocarbons –through hydrophobicity essay of the cell surface–, the self-aggregation capacity, congregation, antimicrobial activity and resistance to 14 antibiotics, were evaluated. Variance analysis was performed on the data with the statistical program INFOSTAT, and to verify the differences among means Duncan's comparison test was used. Fifteen strains grew at different pH and decreased their growth at values equal or lower than 5 and at the optimum temperature of the vaginal ecosystem of the cows, and had high growth capacity at 24 h. The strains LvB-38, 39, 42, 45, 46, 52, 54 and 90 showed capacity of adherence to hydrocarbons (toluene and xylene), higher than 80 %; self-aggregated over 50 %; co-aggregated with *Escherichia coli* (Migula) Castellani and Chalmers, *Staphylococcus aureus* Rosenbach and *Klebsiella* spp., over 75 %; inhibited the growth of these pathogens due to the presence of acids; and were sensitive to ten antimicrobial drugs. It is concluded that the eight selected strains can be considered as candidates to be probiotics, for their utilization in the prevention of urogenital disorders of cows.

Keywords: acid-lactic bacteria, cattle, hydrophobicity

Introduction

Since the last century new biotechnological products were developed and used in intensive animal production systems, in order to decrease losses in meat and milk production, as well as to prevent the contamination of these foodstuffs. With these purposes probiotics are developed, which are living microorganisms, mainly from the *Lactobacillus* genus, which supplied in small doses favor the microbial balance, at the intestine as well as the vagina level. Such products do not cause the microbial resistance problems or residual effect produced by antibiotics, so that they contribute to the promotion of sustainable and ecological animal husbandry.

In the world products with probiotic effects are commercialized, among which the following stand out for their efficacy: ProBiotics®, Biomin®, Biomax **5**® and HYDROYEAST®; nevertheless, there are few reports of the use of vaginal probiotics in dairy cows to prevent uterine disorders. These infections are related to the excessive growth of pathogen bacteria during the first three weeks after calving, which causes a decrease in milk production (Sheldon *et al*., 2006). Leccese *et al*. (2012) consider that these alterations can become systemic infections and affect the fertility or pregnancy of the cow. However, if the beneficial microbiota, mainly formed by lactobacilli, is established, these bacteria can adhere to the epithelial cells and produce antimicrobial substances such as lactic acid, acetic acid, hydrogen peroxide and bacteriocins, which prevent the proliferation of pathogens (Nazef *et al*., 2008).

Lactobacilli represent the most abundant group of lactic-acid bacteria (LAB) in nature and are predominant in the human vaginal tract and in the vaginal tract of some homeothermic animals. They are developed in ecosystems that contain fermentable sugars, vitamins, hydrolyzed protein products, low oxygen tension, among other factors (Orla-Jensen, 1917; Falentin *et al*., 2016). This group is called «guardians of the vagina», and contributes to maintain the adequate microbial balance in such ecosystem (De Gregorio *et al*., 2012; Wang *et al*., 2013). Hence the objective of the study was to evaluate the *in vitro* probiotic potential of 28 *Lactobacillus* spp. strains isolated

from the vagina of creole dairy cows of the Manabí region, Ecuador, for their utilization as probiotic in cows with urogenital disorders.

Materials and Methods

Biological material. Samples were taken from the cervix and the vagina of 15 creole dairy cows of the Manabí region, for the isolation of strains with probiotic potential, specifically of *Lactobacillus* spp.; with these 28 strains the trial was conducted. They were incorporated to the strain bank of the molecular biology laboratory of the Superior Agricultural Polytechnic School of Manabí, and preserved in milk (10 %) and glycerol (20 %) under cryo-conservation conditions at -80 ºC, according to the recommendations made by Vera (2013).

Selection of *Lactobacillus* spp. strains with *in vitro* probiotic potential

Determination of pH. The 28 selected strains were separately cultivated in tubes with 10 mL of MRS (Mann Rogosa Sharpe) broth (De Mann *et al*., 1960), at 37 ºC during 24 h, according to the methodology proposed by Rondón (2009). From each replication samples were taken to measure the pH of the cultures at 24 h, with a digital pH meter (Sartorius Meter PP-25).

Survival at different pH and temperature

pH. The isolated strains were cultivated in MRS broth, whose pH was adjusted to 6, 8 and 9 with NaOH at 2 %, and were replicated three times. The cultures were incubated at 37 ºC during 24 h. Growth was measured through the determination of absorbance (A_{560nm}).

Temperature. The strains were cultivated in MRS broth and were incubated at 38, 39 and 40 ºC during 24 hours. The growth was measured through the same procedure used for pH.

Growth capacity. To determine whether there were differences among the strains in the growth capacity at 0 and 24 hours, a completely randomized design was used with 18 x 2 factorial arrangement, with three repetitions. The strains were cultivated for 18 h (10 Log CFU.mL^{-1}) in MRS broth with pH 6,5 at 37 ºC. Afterwards, they were inoculated at a rate of 1:10 (v/v) in flasks with 45 mL of the same medium and were incubated at 37 ºC during 24 hours under static conditions. The count of *Lactobacillus* spp. was carried out by the method of seriated dilutions, and the plates were incubated at 37 ºC under anaerobic conditions during 48 h.

Cell surface hydrophobicity assay (CSH). The SCH was determined by the water-hydrocarbon biphasic method MATH (microbial adhesion to hydrocarbons test), according to the methodology proposed by Pérez *et al*. (1998). The cell surface hydrophobicity was calculated with the following formula: % $H = (A_0 - A)/A_0$ x 100, where A_0 and A represent the optical density (OD) before and after the extraction with toluene and xylene, respectively. The strains that showed more than 80 % of hydrophobicity were taken as selection criterion.

Self-aggregation capacity. To determine the self-aggregation capacity the method proposed by Kos *et al*. (2003) was used, and for such purpose all the *Lactobacillus* spp. strains that reached the above-indicated hydrophobicity percentage were chosen. They were evaluated during 5 h of incubation, at room temperature. Every hour 1 mL of the top layer was extracted and transferred to tubes that contained 3 mL of PBS buffer. The absorbance of this mixture was measured, and the self-aggregation percentage was determined through the formula: $A = 1\text{-} (A_t/A_0)$ x 100, where A_t represents the OD at the different hours: 1, 2, 3, 4 y 5, and A_0, the OD at zero hour.

Co-aggregation level. The technique described by Orlowski and Bielecka (2006) was used. The wild strains of *E. coli, S. aureus* and *Klebsiella* spp. were isolated from the vaginal ecosystem of sick cows and were identified in the microbiology laboratory of the Superior Agricultural Polytechnic School of Manabí. The strains that had more than 80 % of hydrophobicity were selected, and the absorbance (A) was determined at 560 nm, before and after 5 h, in the pure cultures of lactobacilli, pathogens and the mixture of both. The incubation occurred at room temperature. To determine the percentage of co-aggregation the following equation was used: % co-aggregation = $[(Ax_t + Ay_t)/2 - A_t$ (x+y)/ $(Ax_i + Ay_i)/2]$ x 100.

Where:

Ax_t: absorbance at 5 h of the pure cultures of *Lactobacillus* spp.

Ay_t: absorbance at 5 h of the pure cultures of *E. coli*, *S. aureus* and *Klebsiella* spp.

A_t $(x + y)$: absorbance of the mixture of *Lactobacillus* spp. + *E. coli, S. aureus* and *Klebsiella* spp.

Ax_i: absorbance of the pure cultures of *Lactobacillus* spp. at the initial time.

Ay_i: absorbance of the pure cultures of *E. coli, S. aureus* and *Klebsiella* spp. at the initial time.

Determination of the antimicrobial activity. The technique of substance diffusion in agar, proposed by Schillinger and Lücke (1989), was used. As indicator strains *E. coli, S. aureus* y *Klebsiella* spp. were utilized, which were inoculated in nutrient broth and incubated in thermostated shaker during 18 h at 37 ºC. The producer strains (lactobacilli) were cultivated in 10 mL of MRS broth at 37 ºC, under static conditions, for 18 h (10 Log CFU mL^{-1}). Samples were taken at 8, 18 and 24 hours; were centrifuged at 15 000 rpm, at 5 ºC during 10 min; and were sterilized through cellulose acetate filters, with 0,22-µm pores (Minisart, Sartorius 600 kPa max). The supernatant was not modified.

Diffusion in agar technique. From the indicator strains cultures 200 µL were taken and were inoculated in tubes with 20 mL of nutrient agar (with 10 % of ion-agar, OXOID), which were poured on plates for their solidification. In each plate that contained indicator strains wells of 5 mm diameter were opened and in them 60 µL of the producer strain samples, positive controls (MRS broth + 1N lactic acid until reaching pH 3) and negative controls (MRS broth pH 6,2) were deposited. The plates were maintained at 5 ºC during 4 h, for a better diffusion of the substances in agar. They were later incubated at 37 ºC between 24 and 48 h, until detecting the growth and appearance of the inhibition halos. The diameter of the halos was measured with a millimeter ruler. The diameter of the wells was subtracted from each value.

Determination of the sensitivity to antimicrobials. The determination of the sensitivity of the selected strains to 14 different antibiotics (NEO-SENSITABS™) was carried out through the disk diffusion method (Bauer *et al*., 1966), in MRS agar at 37 ºC under anaerobic conditions, due to the nutritional and physiological demands of lactobacilli. The presence of sensitivity was observed when the inhibition halos were detected. The antibiotic test was conducted in duplicate.

Statistical analysis. A variance analysis was performed on the data with the statistical program INFOSTAT version 1 (Balzarini *et al*., 2001). The differences among means were verified through Duncan's (1955) comparison test.

Results and Discussion

From the 28 strains with characteristics of the lactobacilli group 15 acidified the medium at pH < 5 (table 1), for which they were selected. LABs have the capacity to produce lactic acid and decrease the pH of the substrate, primordial characteristic within the group of lactobacilli with probiotic capacity. Similar results were obtained by Vallejo *et al.* (2008) when evaluating 20 strains of lactobacilli isolated from sheep cheese, 10 of which survived to acid pH conditions. Rondón (2009) isolated 75 strains from chicken cecum, and from them only 42 decreased the pH; while Sánchez *et al*. (2011) isolated a total of 24 strains from the vagina of healthy women and only 16,6 % withstood acid pH. On the other hand, Vera (2013) isolated 54 strains from the vagina of cows and 17 strains acidified the medium.

Table 1. Acidification of the culture medium at 24 hours

Strain	pH	Strain	pH
21 LvB	5,40^{cd}	49 LvB	5,20^{c}
23 LvB	5,70^{d}	50 LvB	3,80^{a}
36 LvB	5,50^{cd}	51 LvB	4,20^{ab}
37 LvB	4,35^{b}	52 LvB	4,00^{ab}
38 LvB	4,31^{b}	53 LvB	4,20^{ab}
39 LvB	4,31^{b}	54 LvB	4,20^{ab}
40 LvB	5,50^{cd}	60 LvB	4,30^{b}
41 LvB	5,90^{d}	62 LvB	5,60^{cd}
42 LvB	4,00^{ab}	63 LvB	6,37^{e}
43 LvB	4,25^{ab}	78 LvB	5,60^{cd}
44 LvB	4,37^{b}	81 LvB	5,60^{cd}
45 LvB	4,32^{b}	84 LvB	5,50^{cd}
46 LvB	4,32^{b}	88 LvB	5,7^{d}
47 LvB	5,50^{cd}	90 LvB	4,35^{b}
EE ±		0,15 **	

a, b, c, d, e: values with different superscripts differ at p <0,05 (Duncan, 1955).

**p<0,01

Table 2 shows that all the strains had good growth, for the different pH as well as for the different temperatures. This indicates that the adaptation of *Lactobacillus* to the conditions of the vaginal ecosystem is an essential characteristic for the survival of such microorganisms in this environment (Redondo-López *et al*., 1990; Bouchard *et al*., 2015). In the selection of strains with probiotic effect in the urogenital tract, it is important to test the resistance capacity to these extreme conditions to consider that these strains are capable of growing and colonizing the vaginal mucosa (Zárate *et al*., 2005; Sánchez *et al*., 2015).

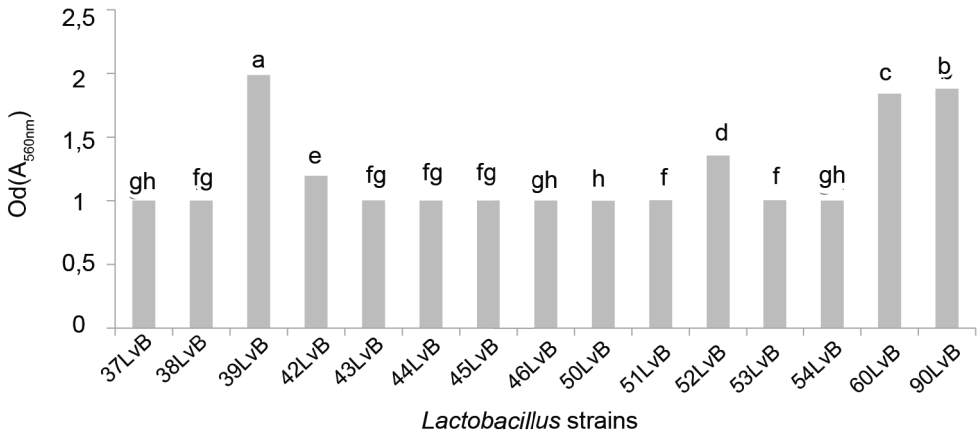
Table 2. Growth (OD A_{560nm}) of the strains isolated at different pH and temperatures in MRS broth, at 24 hours

Strain	38 ºC			39 ºC			40 ºC		
	pH 6	pH 7	pH 8	pH 6	pH 7	pH 8	pH 6	pH 7	pH 8
37	1,36^{d}	0,29^{def}	0,20^{c}	1,90^{ab}	0,24^{f}	0,21^{l}	1,32^{abcde}	0,17^{c}	0,14^{c}
38	1,96^{a}	1,59^{bcdef}	1,45^{bc}	1,94^{b}	1,54^{b}	1,30^{f}	0,53^{bcde}	1,45^{bcd}	1,23^{c}
39	1,97^{a}	1,55^{a}	1,33^{ab}	1,95^{a}	1,91^{a}	1,09^{g}	1,52^{e}	1,45^{a}	1,43^{ab}
42	1,68^{c}	1,58^{ab}	1,56^{ab}	1,94^{a}	1,90^{a}	1,86^{b}	1,63^{abc}	1,60^{ab}	1,52^{abc}
43	1,43^{d}	0,62^{abcde}	0,55^{abc}	1,88^{ab}	0,65^{de}	0,71^{j}	1,53^{abc}	0,50^{ab}	0,50^{abc}
44	1,26^{e}	0,14^{ef}	0,33^{c}	1,94^{a}	0,91^{c}	0,85^{i}	1,68^{abc}	0,61^{bcd}	0,66^{bc}
45	1,32^{e}	0,30^{f}	0,63^{c}	1,34^{cd}	0,51^{e}	0,46^{k}	1,69^{a}	0,59^{abcd}	0,60^{bc}
46	1,76^{b}	1,94^{abcdef}	1,83^{abc}	1,46^{c}	1,73^{ab}	1,62^{e}	1,54^{ab}	1,52^{abcd}	1,51^{abc}
50	0,86^{f}	0,61^{abc}	0,51^{ab}	0,34^{f}	0,03^{f}	0,18^{m}	0,63^{abcde}	0,57^{abc}	0,48^{abc}
51	0,72^{g}	0,60^{def}	0,61^{c}	0,17^{e}	0,04^{f}	0,07^{n}	0,97^{e}	0,82^{abcd}	0,81^{c}
52	1,49^{d}	1,43^{abcdef}	1,41^{abc}	1,22^{d}	1,07^{cd}	1,05^{h}	1,04^{cde}	1,03^{abcd}	0,01^{c}
53	1,49^{d}	1,41^{abcd}	1,51^{a}	1,77^{b}	1,73^{ab}	1,63^{b}	1,51^{de}	1,53^{abc}	1,68^{c}
54	1,50^{d}	1,41^{abcd}	1,51^{a}	1,85^{ab}	1,66^{b}	1,73^{c}	1,49^{abcd}	1,58^{a}	1,68^{a}
60	0,34^{h}	0,32^{abcde}	0,29^{abc}	0,98^{d}	0,61^{de}	0,46^{k}	0,64^{abcde}	0,64^{ab}	0,60^{abc}
90	1,48^{d}	1,52^{cdef}	1,69^{abc}	1,92^{ab}	1,93^{a}	1,95^{a}	1,53^{de}	1,69^{abcd}	1,88^{abc}

a, b, c, d, e, f, g, h, i, j, k, l, m, n: values with different superscripts differ at $p < 0,05$ (Duncan, 1955).

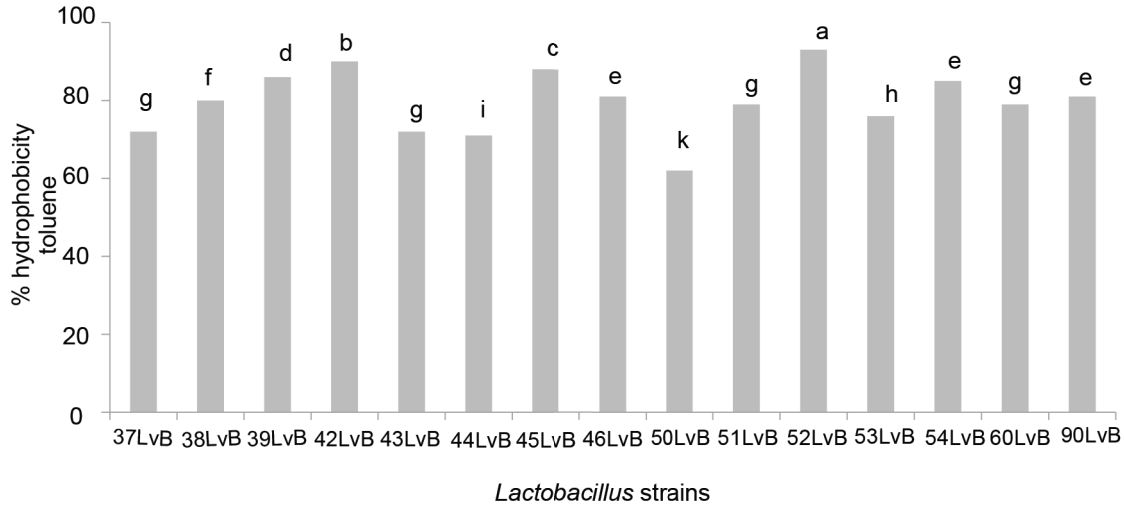
Figure 1 shows the growth of the 15 selected strains in MRS broth, with pH 6,5 at 37 ºC. All of them reached high values, property that should characterize the probiotic strains so that they are capable of becoming established and achieving a high population in the vaginal mucosa. Similar results were obtained by Brolazo *et al.* (2009) when evaluating 37 *Lactobacillus* spp. strains from women's vagina, with high growth capacity and were the ones that acidified pH the most. On the other hand, Sánchez *et al*. (2011) obtained 24 *Lactobacillus* spp. strains, also from women's vagina, from which nine showed the best probiotic characteristics.

Figures 2 and 3 show the results of adherence of the lactobacilli to the organic solvents toluene and xylene; only the strains 38LvB, 39LvB, 42LvB, 45LvB, 46LvB, 52LvB, 54LvB, 60LvB and 90LvB reached hydrophobicity values higher than 80 %.

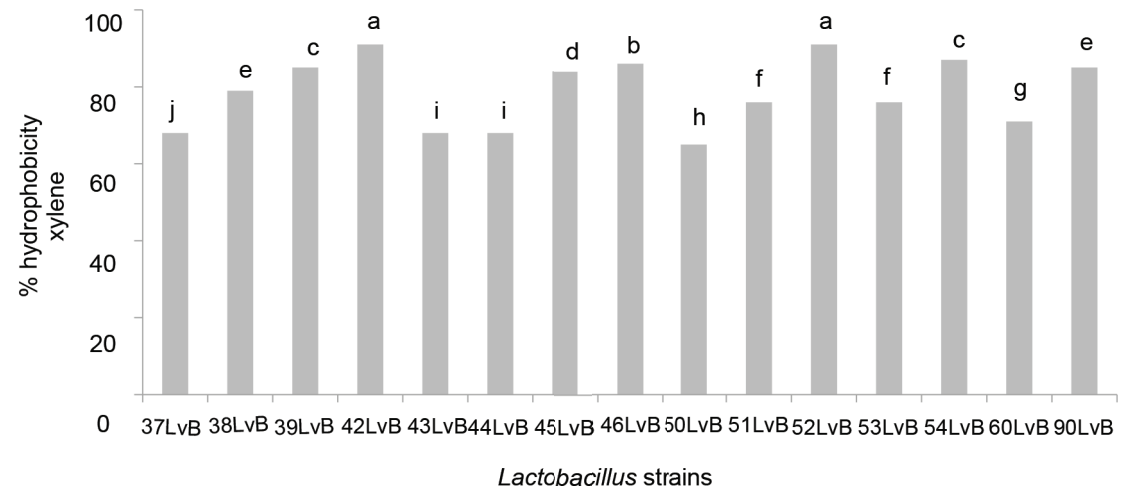


a, b , c, d, e, f, g, h: columns with different letters differ for $p < 0,001$ (Duncan, 1955).

Figure 1. Growth capacity of Lactobacillus spp. strains in MRS broth, at 24 h.



a, b, c, d, e, f, g, h, i: columns with different superscripts differ at p <0,05 (Duncan, 1955).
Figure 2. Performance of hydrophobicity in the presence of toluene.



a, b, c, d, e, f, g, h, i, j: columns with different superscripts differ at p < 0,05 (Duncan, 1955).
Figure 3. Performance of hydrophobicity in the presence of xylene

The adherence capacity of bacteria to the vaginal tract epithelium involves different mechanisms, among which the presence of adhesins on the surface of bacterial cells stands out. Adhesins are mostly proteins that can bind to the carbohydrates found in the glycocalyx of epithelial cells; these carbohydrates work as anchoring sites for bacteria (Savage, 1992; Jewell *et al.*, 2015). Similar results were obtained by Vallejo *et al.* (2008), because the lactobacillus strains isolated from sheep milk cheese reached high hydrophobicity percentages.

Sánchez *et al.* (2011), in strains isolated from human vagina, found only nine with the best characteristics in hydrophobicity tests. This property is a measure that evaluates the adherence capacity of lactobacilli to the epithelium or vaginal mucosa, and consists in nonspecific physical interactions between two surfaces. Such selection criterion is the most important, because, according to Tuomola *et al.* (2001) and Rodrigues *et al.* (2015), without adherence the concentration of probiotics would be low and their effect, insufficient.

In order to determine the self-aggregation capacity, the strains that reached more than 80 % of hydrophobicity were selected (38LvB, 39LvB, 42LvB, 45LvB, 46LvB, 52LvB, 54LvB and 90LvB). Figure 4 shows the sedimentation values of the eight strains that presented phenotypes of strong self-aggregation, measured during an incubation period of 5 hours; from them, the strains 38LvB and 52LvB exceeded 80 %, followed by 42LvB, 45LvB, 46LvB and 54LvB which reached between 60 and 70 %.

According to García *et al*. (2007), self-aggregation in bacteria can be defined as the phenomenon of aggregation among cells from the same strain. The self-aggregation capacity is related to the components of the cell surface, property that was not affected after washing and suspending the cells in PBS buffer. The self-aggregation of the probiotic strains seems to be necessary for the adhesion to epithelial cells to occur (Kos *et al*., 2003). The adhesion of probiotics to intestinal cells and the vaginal mucosa is also considered very important in the stimulation of the immune system, and acts as barrier against pathogen microorganisms (Bouridane *et al*., 2016).

Co-aggregation is the aggregation that occurs between different species. Table 3 shows the co-aggregation percentage reached by the *Lactobacillus* spp. cells to the cell walls of *E. coli*, *S. aureus* and *Klebsiella* spp., in the nutrient broth. All the evaluated strains showed a co-aggregation higher than 50 %, except LvB-52 against *E. coli* (18 %). The aggregation capacity is an important characteristic for any study of microbial interactions, and there is an association among the ability of lactobacilli to adhere to the vaginal epithelium, aggregation and hydrophobicity (Williams *et al*., 2007; Sánchez *et al*., 2015).

The fact that the evaluated bacteria showed high co-aggregation indicates that these lactobacilli can have an important effect on the regulation and balance of the vaginal ecosystem, and also on the stimulation of the immune system (De Gregorio *et al*., 2012; Sandes, 2013). Such authors as Swartz *et al*. (2014) and Minuti *et al*. (2015) describe that the protective effect of these microorganisms occurs through two mechanisms: antagonism and toxin production.

Table 4 shows the results of the antimicrobial activity, from 8 to 24 h, the strain 38LvB produ-

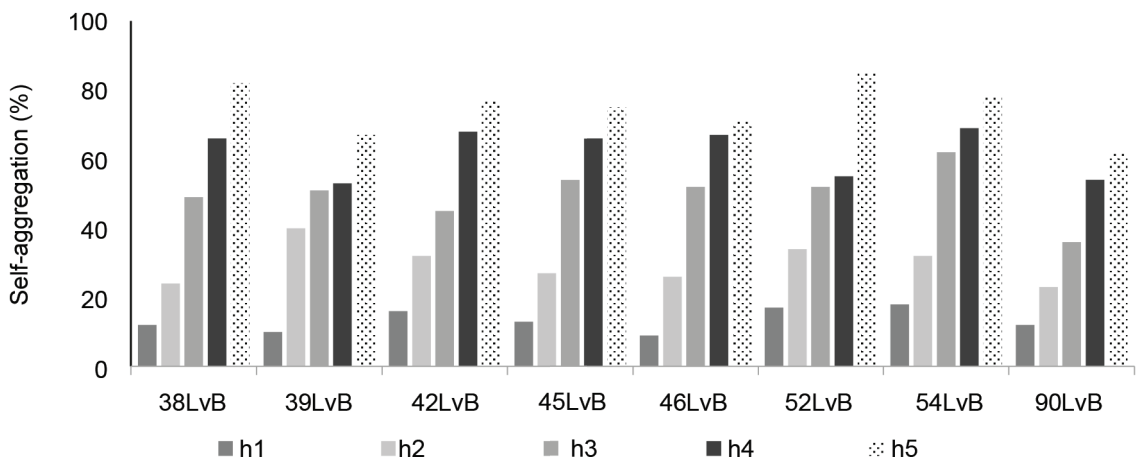


Figure 4. Self-aggregation capacity in selected *Lactobacillus* spp. strains.

Table 3. Co-aggregation of *Lactobacillus* spp. cells against indicator bacteria.

Indicator strain	% of co-aggregation							
	38LvB	39LvB	42LvB	45LvB	46LvB	52LvB	54LvB	90LvB
E. coli	65^{d}	85^{a}	72^{b}	58^{e}	57^{f}	18^{h}	42^{g}	66^{c}
S. aureus	62^{e}	85^{b}	58^{f}	80^{c}	57^{g}	86^{a}	48^{h}	65^{d}
Klebsiella spp.	66^{e}	85^{a}	77^{b}	75^{c}	60^{g}	70^{d}	63^{f}	77^{b}

a, b, c, d, e, f, g, h: values with different superscripts differ at $p < 0,05$ (Duncan, 1955).

Table 4. Antimicrobial activity of the indicator strains

Indicator strain	Producer lactobacilli strains by presence of acids (halo, in mm)							
	38LvB	39LvB	42LvB	45LvB	46LvB	52LvB	54LvB	90LvB
Hour 8								
S. aureus ATCC-29213	NI	NI	NI	NI	NI	NI	NI	NI
E. coli ATCC-25922	14,0^{f}	9,65^{a}	9,89^{b}	11,45^{e}	10,75^{c}	11,45^{e}	10,74^{c}	11,25^{d}
E. coli spp.	13,7^{g}	5,7^{b}	5,26^{a}	11,67^{e}	5,26^{a}	13,43^{f}	6,70^{c}	9,60^{d}
Klebsiella spp.	8,30^{g}	7,30^{c}	7,03^{b}	8,03^{e}	7,70^{d}	8,27^{f}	6,65^{a}	8,90^{h}
Hour 18								
S. aureus TCC-29213	NI	NI	NI	NI	NI	NI	NI	NI
E. coli ATCC-25922	10,53^{g}	8,27^{a}	8,3^{b}	10,00^{e}	9,73^{d}	10,30^{f}	9,65^{c}	10,0^{e}
E. coli spp.	9,17^{h}	8,70^{e}	7,70^{b}	8,90^{f}	8,30^{d}	9,03^{g}	7,68^{a}	8,20^{c}
Klebsiella spp.	13,43^{f}	12,03^{cd}	12,03^{c}	12,67^{de}	12,03^{cd}	13,37^{ef}	7,50^{a}	9,84^{b}
Hour 24								
S. aureus ATCC-29213	NI	NI	NI	NI	NI	NI	NI	NI
E. coli ATCC-25922	13,80^{h}	13,27^{f}	12,03^{d}	12,30^{e}	11,03^{c}	13,73^{g}	0,25^{a}	10,30^{b}
E. coli spp.	10,70^{f}	10,00^{e}	9,30^{a}	9,70^{cd}	9,70^{cd}	10,53^{f}	9,80^{d}	9,50^{ab}
Klebsiella spp.	17,99^{f}	16,03^{c}	16,33^{c}	17,03^{e}	16,70^{d}	18,03^{f}	8,10^{a}	9,00^{b}

a, b, c, d, e, f, g, h: values with different superscripts in the same column differ at $p < 0,05$ (Duncan, 1955).

ced higher inhibition halos ($p \leq 0,001$) in the three indicator microorganisms. In this trial the unmodified supernatant was used, that is, all the possible substances that inhibited growth were present. The highest inhibition halos were appreciated in the *E. coli* ATCC-25922 and wild *E. coli* strains, at 8, 18 and 24 h, followed by *Klebsiella* spp.; however, *S. aureus* did not show inhibition.

It is known that the beneficial vaginal microbiota participates in the maintenance of the ecological balance of the area and exerts resistance to the colonization by pathogens that cause bovine metritis, as reported by Sánchez *et al.* (2011) and Zhang *et al*. (2015). Studies conducted by Wang *et al.* (2013) and Otero *et al.* (2006) suggest that, due to its complexity, the vaginal ecosystem of dairy cows, which is similar to the human vaginal ecosystem, should continue to be studied.

Table 5 shows that the eight strains were resistant to four antibiotics: Amikacin, Azithromycin, Vancomycin, and Neomycin. In this sense, Souza *et al*. (2007) reported that, when performing antibiograms to *Lactobacillus* strains against 12 antibiotics, all of them were resistant to Vancomycin and Nalidixan, and that a high percentage of the strains was resistant to Gentamicin, results which were very similar to the ones in this study. In the specific case of Vancomycin, it was proven that the resistance to this antibiotic is intrinsic of the members of the *Lactobacillus* genus and that it is genetically conditioned (Danielsen and Wind, 2003; Wage, 2003).

Sánchez *et al*. (2011), when evaluating 17 antimicrobial drugs against *Lactobacillus* strains from women's vagina, found that seven of them were resistant. It is known that the misuse of antibiotics can cause bacteria that were not resistant to reach accidentally this condition, because they capture the DNA of the resistant ones (Martín *et al*., 2008). Nevertheless, it is necessary to use specific genetic methods to depurate the presence of plasmids with resistance to antimicrobials, such as the hybridization by PRC (Tenover and Rasheed, 1999; Martina *et al*., 2015).

Hence the importance of the use of probiotics as alternative to prevent urogenital diseases in the cows, which would allow to restrict the use of antibiotics only to prophylactic cases, in order to contribute to the decrease of the resistance to the antimicrobial drugs which also affect human beings.

Conclusions

The *Lactobacillus* spp. strains 38LvB, 39LvB, 42LvB, 45LvB, 46LvB, 52LvB, 54LvB and 90LvB, isolated from the cervix and the vagina of healthy creole cows, have potential to be used in the prevention of infections of the reproductive tract of the cow, because they showed, *in vitro*, resistance to

Table 5. Resistance of the strains to different antibiotics.

Antimicrobial drug	(µg)	*Lactobacillus* strains from vagina of dairy cows							
		38	39	42	45	46	52	54	90
Kanamycin	100	S	S	S	S	S	S	S	S
Amikacin	30	R	R	R	R	R	R	R	R
Ampicillin	33	S	S	S	S	S	S	S	S
Azithromycin	15	R	R	R	R	R	R	R	R
Erythromycin	200	S	S	S	S	S	S	S	S
Amoxicillin	30	S	S	S	S	S	S	S	S
Ciprofloxacin	0,5	S	S	S	S	S	S	S	S
Streptomycin	100	S	S	S	S	S	S	S	S
Vancomycin	5	R	R	R	R	R	R	R	R
Apramycin	40	S	S	S	S	S	S	S	S
Bacitracin	40	S	S	S	S	S	S	S	S
Neomycin	120	R	R	R	R	R	R	R	R
Lincomycin	19	S	S	S	S	S	S	S	S
Estreotomycin	200	S	S	S	S	S	S	S	S

S: sensitive, R: resistant

acid pH, broad spectrum of antimicrobial activity and high capacity of adhesion to the epithelium of the vaginal mucosa.

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