



## Evaluation of the Probiotic Profile of the *Lactobacillus Acidophilus* Used in Pharmaceutical and Food Applications

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**ABSTRACT.** *Lactobacillus acidophilus* used in three different applications, compounding pharmacies (LA1), fermented dairy (LA2), and allopathic compoundings (LA3) were tested to evaluate the existence of significant differences between them and in different growth conditions. In the evaluation of resistance to different commercial use antibiotics, all strains were sensitive to the antibiotics ampicillin, chloramphenicol, doxycycline, and tetracycline. LA1 was considered moderately sensitive (MS) to erythromycin and LA3 was MS to clindamycin and erythromycin. LA3 was classified between MS to resistant to erythromycin. All three strains were resistant to gentamicin. When evaluating acid pH resistance, the three origins presented similar behavior, with a decrease in cell viability at pH 2, maintaining constant viability at pH 3 and 4. In the test of resistance to the gastrointestinal tract conditions and hydrophobicity, LA2 presented better results. The three strains showed production of inhibitory compounds against pathogenic bacteria and deconjugated tauroconjugated bile salts (TDCA). It was concluded that, depending on the origin, *Lactobacillus acidophilus* may present different behaviors that will determine its growth and, consequently, its action *in vivo*. Due to the practicality of access, economy, and the satisfactory results in the tests performed, LA2 can be considered the strain of choice among those studied.

**Keywords:** intestinal health, immunity, resistance to pathogens.

## Avaliação do perfil probiótico de *Lactobacillus acidophilus* utilizado em aplicações farmacêuticas e alimentares

**RESUMO.** *Lactobacillus acidophilus* utilizados em três diferentes aplicações, sendo: farmácias de manipulação (LA1), em produtos lácteos fermentados (LA2) e em formulações alopatóicas (LA3) foram testados para avaliar a existência de diferenças significativas entre elas e em diferentes condições de crescimento. Para o teste de avaliação da resistência aos diferentes antibióticos de uso comercial todas as estirpes de LA foram sensíveis aos antibióticos ampicilina, cloranfenicol, doxiciclina e tetraciclina. LA1 foi considerada moderadamente sensível (MS) a eritromicina e LA3 MS a clindamicina e eritromicina. LA3 enquadrou-se na classificação entre MS a resistente (R) para eritromicina. As 3 cepas de LA foram resistentes a gentamicina. Na avaliação à resistência em pH ácido, as três origens apresentaram comportamento similar, com diminuição da viabilidade celular em pH 2, mantendo constante a viabilidade em pH 3 e 4. No teste de resistência às condições do trato gastrointestinal e hidrofobicidade LA2 apresentou os melhores resultados. As 3 estirpes apresentaram produção de compostos inibitórios frente a bactérias patogênicas e desconjugaram sais biliares tauroconjugados (TDCA). Concluiu-se que, dependendo da origem, o *Lactobacillus acidophilus* pode apresentar diferentes comportamentos que poderão determinar o seu crescimento e consequentemente sua ação *in vivo*. Pela facilidade, praticidade de acesso, economia e resultados satisfatórios nos testes realizados, LA2 pode ser considerada a estirpe de escolha entre as estudadas.

**Palavras-chaves:** saúde intestinal, imunidade, resistência a patógenos.

### Introduction

Probiotics are considered as GRAS ingredients (generally recognized as safe) (Mattia & Merker, 2008), and their consumption reduces the viable number of pathogens while strengthening the body's natural defenses (Bertazzoni-Minelli & Benini, 2008;

Madureira et al., 2008; Larsen, Michaelsen, Pærregaard, Vogensen, & Jakobsen, 2009; Savard et al., 2011). Hence, they help boost the immune system and thus lower the risk of gastrointestinal diseases, cancer, diabetes, and elevated serum cholesterol levels while improving digestion itself (De Vrese & Schrezenmeir, 2008; Kumar et al., 2012).

The ingestion of probiotic microorganisms may be in the form of pharmaceutical preparations such as powdered compounds, tablets, or capsules, or as yogurts and other fermented foods. These products may contain only one or several distinct species of microorganisms (Nagpal et al., 2012).

The use of *Lactobacillus acidophilus* as a probiotic provides benefits such as reduction in abdominal pain or discomfort in patients with irritable bowel syndrome (Sinn DH, Song JH, Kim HJ, et al., 2008), reduction in total cholesterol levels, LDL-c, and triacylglycerols in animals (Park et al., 2007; Park et al., 2008) and a reduction in insulin resistance (Andreasen et al., 2010).

The ability of probiotic microorganisms to survive and develop in the host strongly influences their probiotic effects. The microorganism that is metabolically stable in the product and survives the passage through the digestive tract with high viability may have beneficial effects when present in the host intestine (Anal & Singh, 2007).

Viability and activity are not the only key factors in the action of a probiotic. The microorganism level must be sufficiently high (Stefe, Alves, & Ribeiro, 2008). In Brazil, to market products containing probiotics, the National Agency of Sanitary Surveillance (ANVISA) requests the submission of an analysis report that proves the minimum viable quantity of the microorganism needed to exercise functional property at the end of the product's shelf life and in the use, storage, and distribution conditions (National Agency of Sanitary Surveillance [ANVISA], 2016).

The lack of information regarding the activity of probiotic microorganisms administered by means of pharmaceutical forms, such as capsules, often called nutraceuticals, and food products of different origins justifies this study, which aims to evaluate the differences in the probiotic profile.

The objective of this article is to compare the probiotic profile of *Lactobacillus acidophilus* of different origins. For this, their growth was evaluated in the presence of commercial use antibiotics, as well as their resistance in conditions that simulate in vitro the gastrointestinal environment. We aimed to investigate the in vitro adhesion abilities of these probiotic cultures, as well as evaluate the production capacity of bile salt hydrolase enzymes and the production of inhibitory compounds with bactericidal characteristics.

## Material and methods

### a. Microorganisms

*Lactobacillus acidophilus* from three different origins were used:

- Origin 1 (LA1): *Lactobacillus acidophilus* from a manipulated supplement, marketed in the form of capsules or sachets to regulate the intestinal microbiota in case of diarrhea or constipation. According to the Aché supplier's specification, each gram of lyophilized product contained  $10^9$  CFU.

- Origin 2 (LA2): *Lactobacillus acidophilus*, used in food formulations as fermented products, kindly provided by SACCO® Brazil. According to the supplier's specification, each gram of lyophilized product contained  $10^{11}$  CFU.

- Origin 3 (LA3): *Lactobacillus acidophilus* of allopathic origin, marketed in dispensing pharmacies, also with the purpose of regulating the intestinal microbiota. According to the supplier's specification, each capsule contained the equivalent of  $10^9$  CFU g<sup>-1</sup>.

### b. Microorganism activation

The microorganisms were placed in 10% (v/v) TSB medium (Tryptic Soy Broth – Acumidia-Maryland) and incubated for 24 hours in an anaerobic jar at  $37 \pm 1^\circ\text{C}$ . After this incubation period, centrifugation was performed for 5 minutes and they were washed three times with a pH 7.0 phosphate-buffered saline solution.

### c. Cellular viability evaluation

Cell viability was determined by the depth seeding technique (Zayed & Winter, 1995) using the MRS agar medium (Man Rogosa and Sharp). The incubation was carried out for a period of 48h, in an anaerobic jar, at  $37 \pm 1^\circ\text{C}$ . The analyses were performed in duplicate, with two repetitions.

For counting, plaques that presented between 30 and 300 colonies were used, and the individual result was multiplied by the respective dilution. The mean dilution results were expressed in CFU g<sup>-1</sup> of product.

### d. Inoculum preparation

To ensure that in all tests performed the number of microbial cells added would be exactly the same, the bacterial inoculum was standardized and the absorbance was equivalent to a standard solution equal to 0.5 on the Mac Farland scale to a wavelength of 625nm, where the desired optical density was between 0.08 to 0.10, which equated to  $1.5 \times 10^8$  CFU (National Committee For Clinical Laboratory Standards [NCCLS], 2015).

#### e. Evaluation of resistance to different commercial use antibiotics

The antimicrobial resistance profile in *Lactobacillus acidophilus* was determined from the antibiogram, which was performed in duplicate, with three repetitions, according to the adapted antimicrobial susceptibility technique by diffusion of the drug in disks (Charteris et al., 1998). The following antimicrobials were tested: Ampicillin (10 µg), Clindamycin (2 µg), Chloramphenicol (30 µg), Doxycycline (30 µg), Erythromycin (15 µg), Gentamicin (10 µg), and Tetracycline (30 µg) (Laborclin, Brazil).

#### f. Resistance in acidic conditions

The *Lactobacillus acidophilus* resistance to hydrochloric acid was tested according to the procedure described by Charteris et al.

#### g. Determination of gastrointestinal transit tolerance

Simulating the conditions of the stomach and small intestine, the viability of the microorganisms was determined against pepsin at pH 2.0 and pancreatin at pH 8.0, respectively, according to the methodology described by Charteris et al. (1998).

#### h. Adherence to cell surface solvents for Lactobacilli test

The hydrophobicity of the bacterial cell surface was evaluated by measuring the microbial adhesion to solvents (MATS), as described by Rosenberg, Gutnick, and Rosenberg (1980) and Pelletier et al. (1997). (2003). The percentage of bacterial adherence to the solvent was calculated as

$$\text{MATS} = \left( \frac{(1 - A1)}{A0} \right) \times 100$$

A0 = Absorbance reading of the cell suspension in KNO<sub>3</sub>.

A1 = Absorbance reading of the aqueous phase, after 30 minutes of exposure to organic solvents.

The isolated were classified as high (66.67 to 100%), medium (33.37 to 66.66%), and low hydrophobicity (0 to 33.33%) as proposed by Nader-Macías, Otero, Espeche, and Maldonado (2008). The results obtained were based on the average of three experiments.

#### i. Antagonistic substances production

To verify the production of antibacterial substances, the multilayer inhibition technique described by Diep, Havarstein, and Nes (1995) was used.

#### j. Bile salt hydrolase activity (BSH)

In the present study, we used the methodology by Tanaka, Hashiba, Kok, and Mierau (2000) based on the deconjugation of TDCA (sodium tauro deoxycholate hydrate - Sigma Aldrich) and GDCA (Glyco deoxy cholic acid monohydrate - Sigma Aldrich) bile salts per *Lactobacillus acidophilus* strains.

#### k. Statistical analyses

For tests of resistance to different commercial use antibiotics, resistance to acid conditions, tolerance to the gastrointestinal tract and hydrophobicity, we applied the variance analysis and the averages of the treatments were compared among themselves by the Tukey test with 5% probability, using the Statistica®7.0 and Minitab 14® programs.

### Results and discussion

#### Cellular viability evaluation

To ensure that the amount of microorganism reported on the product packaging was consistent, the microbial cell count viability test was performed. Table 1 shows the concentrations of the microorganisms under study.

**Table 1.** *Lactobacillus acidophilus* viability according to origin.

Origin	CFU <sup>-1</sup> log	Declared reliabil*†
LA1	9.20	9
LA2	11.16	11
LA3	9.06	9

\*Viability declared in the report and/or package insert provided by the manufacturer.

The results obtained are in accordance with the information presented by the manufacturers /suppliers.

Barreto et al. (2003) evaluated the viability of *Lactobacillus acidophilus*, bifidobacteria, and total bacteria in 177 samples from 15 brands of probiotic products marketed in Brazil from January to August 2001. In their study, they found that most products had a total count of viable microorganisms above 10<sup>7</sup> CFU g<sup>-1</sup>.

#### Evaluation of resistance to different commercial use antibiotics

The results presented in Table 2 demonstrate that the cultures with inhibition halos greater than the reference values were considered as sensitive to antibiotics. Cultures that presented inhibition zones smaller than the reference values were considered moderately sensitive and resistant. *Lactobacillus* species are generally sensitive to inhibitors of

protein synthesis, such as chloramphenicol, erythromycin, clindamycin, and tetracycline, and more resistant to aminoglycosides (neomycin, kanamycin, streptomycin, and gentamicin) (Darsanaki, Aliabadi, & Chakoosari, 2013). This is partially presented in our results, where all lactobacilli were inhibited by chloramphenicol, doxycycline, and tetracycline. And also by the cell wall synthesis inhibitor (ampicillin). However, when analyzing the inhibition halo of the antibiotic erythromycin, we observed that LA1 and LA2 are in the moderately sensitive range, while LA3 is resistant. Similarly, Thumu, and Halami (2012) found that *Lactobacillus plantarum* isolated from dairy products was resistant to erythromycin.

When analyzing the antibiotic clindamycin, LA2 was in the range between moderately sensitive and sensitive. This fact can be explained by the use of strains of different origins. Antimicrobial susceptibility evaluation studies in bacteria of the genus *Lactobacillus*, conducted by Danielsen and Wind (2003), indicated that the level of susceptibility is dependent on the origin of the strains.

Resistance was observed for the antibiotic gentamicin (aminoglycoside) in the three microorganisms tested. This fact occurs due to the inhibition of antibiotic transport in the bacterial cell

since the entry of this drug into prokaryotic cells is O<sub>2</sub>-dependent. This explains the natural resistance of strictly anaerobic bacteria to this antibiotic (Gueimonde, Sánchez, Los Reyes-Gavilán, & Margolles, 2013).

It is also known that aminoglycosides are more widely used against Gram-negative and non-Gram-positive enteric bacteria such as the genus *Lactobacillus* (Gueimonde et al., 2013).

Many resistance mechanisms in probiotic cultures are attributed to complex intrinsic characteristics such as cell wall structure or metabolic properties, and impermeability is the most frequently observed intrinsic resistance mechanism (Charteris et al., 1998).

At the same time, although many strains of lactic bacteria, particularly *Lactobacillus* spp., are resistant to certain antibiotics, this resistance is not normally mediated by plasmids and is not transmissible (Stefe et al., 2008). This feature is beneficial as it enables the use of *Lactobacillus* in therapy associated with antibiotics.

#### Resistance to acidic conditions

In order to reach the intestine and ensure its functionality, probiotic bacteria must have resistance to gastric juice as one of its characteristics, as shown in Table 3.

**Table 2.** *Lactobacillus acidophilus*' sensitivity to antimicrobials according to the origin demonstrated by the mean diameter of the inhibition halos, followed by the standard deviation.

Antibiotic	Inhibition Halo mean diameter (mm)			*R	Sensitivity Profile*	
	LA1	LA2	LA3		*MS	*S
Ampicillin	34.00±0.82 <sup>a</sup>	21.25 ±2.06 <sup>c</sup>	29.20 ±1.89 <sup>b</sup>	≤12	13-15	≥ 16
Clindamycin	16.75±2.88 <sup>a</sup>	12.00±1.15 <sup>a</sup>	16.75±1.15 <sup>a</sup>	≤8	9-11	≥12
Chloramphenicol	29.50±0.57 <sup>ac</sup>	27.25±0.95 <sup>c</sup>	31.25±2.75 <sup>ab</sup>	≤13	14-17	≥18
Doxycycline	23.50±2.51 <sup>c</sup>	32.50±2.08 <sup>ad</sup>	31.75±0.5 <sup>bd</sup>	≤14	15-18	≥19
Erythromycin	15.70±2.21 <sup>a</sup>	16.75±0.95 <sup>a</sup>	13.50±2.38 <sup>a</sup>	≤13	14-17	≥18
Gentamicin	08.25±0.5 <sup>a</sup>	09.25±0.5 <sup>a</sup>	09.50±0.57 <sup>a</sup>	≤12	—	≥13
Tetracycline	20.75±1.5 <sup>b</sup>	31.50±1.9 <sup>a</sup>	31.00±1.82 <sup>a</sup>	≤14	15-18	≥19

\*Averages followed by the same letter in the line do not differ statistically from each other by the Tukey test ( $p > 0.05$ ). The results are expressed as R (resistant), S (susceptible) or MS (moderately susceptible) (Charteris et al., 1998a).

**Table 3.** Average growth (CFU g<sup>-1</sup> log) followed by the standard deviation of microorganisms exposed to hydrochloric acid (HCl P.A.) for up to 3 hours.

ORIGIN	Exposure time (hours)	Exposure to different pH (CFU g <sup>-1</sup> log)			
		pH 2	pH 3	pH 4	control pH (6.5)
LA1	0	5.46±0.15 <sup>aA</sup>	5.85±0.06 <sup>aA</sup>	5.54±0.34 <sup>aA</sup>	5.62±0.32 <sup>bA</sup>
	1	1.90±0.21 <sup>bA</sup>	5.67±0.32 <sup>aA</sup>	5.97±0.17 <sup>aA</sup>	6.21±0.15 <sup>bA</sup>
	3	1.52±0.11 <sup>cA</sup>	5.78±0.46 <sup>aA</sup>	6.01±0.44 <sup>aA</sup>	7.18±0.11 <sup>aA</sup>
LA2	0	6.08±0.14 <sup>aA</sup>	5.87±0.10 <sup>aA</sup>	6.06±0.09 <sup>aA</sup>	6.12±0.03 <sup>aA</sup>
	1	2.07±0.38 <sup>bA</sup>	5.54±0.09 <sup>aA</sup>	6.16±0.02 <sup>aA</sup>	6.30±0.19 <sup>aA</sup>
	3	1.99±0.01 <sup>bA</sup>	4.25±0.07 <sup>biB</sup>	6.05±0.22 <sup>aA</sup>	6.80±0.31 <sup>aA</sup>
LA3	0	5.78±0.46 <sup>aA</sup>	5.57±0.39 <sup>aA</sup>	5.52±0.06 <sup>aA</sup>	5.83±0.12 <sup>cA</sup>
	1	2.11±0.44 <sup>bA</sup>	4.83±0.24 <sup>aA</sup>	5.89±0.30 <sup>aA</sup>	6.27±0.16 <sup>ciA</sup>
	3	1.90±0.14 <sup>bA</sup>	4.66±0.15 <sup>abB</sup>	6.27±0.37 <sup>aA</sup>	6.87±0.18 <sup>aA</sup>

\* Equal lowercase letters in the same column and uppercase letters on the same line correspond to the same averages by the Tukey test ( $p > 0.05$ ).

The medium at pH 2.0 over the incubation time caused a gradual reduction in the number of viable cells, but at the end of 3 hours, viable cells remained.

At pH 3.0, there was no significant difference ( $p > 0.05$ ) in the viable cell count for the LAs evaluated.

The ability to survive at pH 3.0 over an approximate period of three hours is a fundamental characteristic for a microorganism to have a probiotic profile, since that it is the stomach's pH after food ingestion and the average permanence time of the food under these conditions (Collado, Isolauri, Salminen, & Sanz, 2009).

It was observed that the microorganisms tested had the same growth characteristic at pH 4.0 and pH 6.5 (control pH) during the incubation period, i.e., there was no significant difference during the incubation time ( $p > 0.05$ ). Although pH 4.0 was considered acid, an increase in cell viability was observed at the end of the exposure time of these microorganisms. This occurred because this pH range (from 4 to 6.5) is considered ideal for the growth of *Lactobacillus* species (Park & Floch, 2007).

The nature of the food affects the transit time through the stomach, but normally the food remains for 2 to 4 hours (Collado et al., 2009), as well as the pH of the gastric contents. These factors affect the action of probiotic bacteria and indicate that the recommendation of ingestion of this microorganism type, whether in the form of capsules or fermented foods, should not be made while fasting, but soon after meals so that their effects are maintained or potentiated.

#### Determination of tolerance to the gastrointestinal tract

In vitro methodologies represent an important way to characterize GT tolerance, since, in addition to guaranteeing reliable results, they are performed more easily than *in vivo* studies (Charteris et al., 1998).

As proposed by Charteris, Kelly, Morelli, and Collins. (1998), we considered tolerant microorganisms for the gastric simulation survival test those that decreased their cellular concentration to a maximum of 30%.

According to the results presented in table 4, the three strains of LA presented a considerable decrease in viability, superior to the recommended 30% for the simulation of gastric conditions, however, it is still possible to observe viable cells after 180 minutes.

**Table 4.** Total viable cells resistant to the gastric tolerance simulation test at different exposure times.

Origin	Exposure to pepsin (CFU mL <sup>-1</sup> log)		
	1 min..	90 min..	180 min..
LA1	7.93 <sup>aA</sup> (±0.02)	5.74 <sup>BB</sup> (±0.08)	3.60 <sup>CC</sup> (±0.05)
LA2	7.94 <sup>aA</sup> (±0.03)	6.04 <sup>bA</sup> (±0.11)	4.66 <sup>aA</sup> (±0.04)
LA3	7.93 <sup>aA</sup> (±0.05)	5.75 <sup>BB</sup> (±0.05)	3.79 <sup>BB</sup> (±0.03)

\* Equal lowercase letters in the same row do not differ from each other for the Tukey Test with  $p > 0.05$ . Equal uppercase letters in the same column do not differ from each other for Tukey's test with  $p > 0.05$ .

We found that, of the three strains, the one that presented the highest number of viable cells at the end of the experiment was LA2 - *Lactobacillus acidophilus* for food applications.

Vizoso Pinto, Franz, Schillinger, and Holzapfel (2006) presented a study where isolated *Lactobacillus* strains from African fermented dairy products as well as human intestinal isolates were identified and investigated in vitro for their functional and technological characteristics as the potential for new probiotic strains. For the test that simulates gastrointestinal conditions, in the passage through the stomach model, five strains identified as *L. plantarum* and two identified as *L. johnsonii* showed good survival.

With respect to intestinal resistance, it is recommended that the microorganism have a reduction of at most 1.5 log of its initial count. As shown in Table 5, all strains remained within this range, and LA2 was the strain that showed the greatest resistance against pancreatin.

**Table 5.** Total viable cells resistant to the gastric transit tolerance simulation test at different exposure times.

Origin	Exposure to pancreatin (CFU mL <sup>-1</sup> log)	
	1 min.	240 min.
LA1	7.87 <sup>aA</sup> (±0.03)	6.57 <sup>BC</sup> (±0.07)
LA2	7.89 <sup>aA</sup> (±0.03)	7.38 <sup>bA</sup> (±0.04)
LA3	7.89 <sup>aA</sup> (±0.04)	6.79 <sup>BB</sup> (±0.02)

\* Equal lowercase letters in the same row do not differ from each other for the Tukey Test with  $p > 0.05$ . Equal uppercase letters in the same column do not differ from each other for Tukey's test with  $p > 0.05$ .

A similar result was found by Pithva, Shekh, Dave, and Vyas (2014). When testing *Lactobacillus*-based commercial fermented products they found that the greatest reduction in strain viability after the simulation of intestinal transit occurred with a mean reduction of 1.3 log.

The incubation time for the gastric treatment (120 min) and intestinal fluid (180 min) test simulates food intake and the passage time from the stomach to the intestine during digestive processes (Cordonnier et al., 2015).

#### Adherence to surface solvents

The hydrophobicity index can be used to predict the adhesion potential of the strains by

facilitating their contact with the hydrophobic surface of the eukaryotic epithelial cells or with the hydrophilic nature of the mucus covering the surface of the epithelium in some specific areas (Nader-Macías, Otero, Espeche, & Maldonado, 2008).

Bacterial adhesion depends in part on reversible or irreversible interactions. The initial and reversible stage is mediated by a complex of physicochemical interactions, including hydrophobicity and loads, which are not considered specific but important properties (Pelletier et al., 1997).

Through the microbial adhesion to solvents test (MATS), it is possible to qualitatively evaluate how polar or apolar the bacterial surface is since it would indicate the potential of adhesion of the probiotic to the apolar surfaces of the intestinal and vaginal epithelium. However, we propose that the test be only the primary indicator for microorganism adhesion (Mangoni et al., 2011).

Cell surface hydrophobicity and hydrophilicity were evaluated by separating cells between the aqueous and organic phases.

According to the classification proposed by Nader-Macías et al. (2008), where the high hydrophobicity is between 66.67 to 100%, average between 33.37 to 66.66%, and low hydrophobicity is 0 to 33.33%, we see that the adhesion of LA1 cells to xylol is in the range of medium adhesion, whereas for the ethyl acetate and chloroform solvent the adhesion may be considered high. The same profile was observed for LA2 and LA3 strains. Among the three strains evaluated, the one with the highest adhesion values was LA2.

In contrast to the results found in this work, when evaluating the cell surface hydrophobicity of isolates from six distinct species of lactobacilli from industrial products, Pelletier et al. (1997) performed the MATS test for xylol, an organic non-polar solvent, and verified that the microorganisms studied were relatively hydrophilic, with solvent adhesion percentages varying between 2.7 and 26.5%.

The highest adhesion value for *L. plantarum* FAbM2 microorganisms was obtained from chloroform with a maximum of 72% (Sathyabama,

Vijayabharathi, Brinda Priyadarisini, Ranjithkumar, & Bruntha, 2012).

A study with *L. plantarum* showed an affinity above 40% for an apolar solvent, generally present with high hydrophobic characteristics (Giarous, Chapot-Chartier, & Briandet, 2009).

According to Giarous et al. (2009), probiotic strains must have a hydrophobic surface because they have a high adhesion capacity to intestinal cells and solid materials.

The ability to adhere to mucosal surfaces of the intestine and subsequent long-term or short-term colonization has been one of the most commonly encountered criteria for the selection of probiotic strains (Lebeer, Vanderleyden, & De Keersmaecker, 2008; Collado et al., 2009).

### Antagonist activity against pathogens

The mechanisms of antibacterial activity in *Lactobacillus* probiotic strains appear to be multifactorial (Castillo, de Moreno de LeBlanc, Galdeano, & Perdígón, 2013) and are due to the presence of bacteriocins and/or organic acids produced by them.

The bacteria tested in the present work presented antagonistic activity for Gram-positive and Gram-negative pathogens.

The three strains of *L. acidophilus* presented inhibitory halos for all the pathogenic microorganisms tested, as follows: *Salmonella* spp., *Escherichia coli*, *Staphylococcus* sp., and *Listeria monocytogenes*. In some plates, a greater number of halos is verified when compared to others, however, the methodology used does not allow the quantification of the activity, only determines presence or absence of inhibition.

The inhibitory effect may be due to the production of  $H_2O_2$ , lactic acid, bacteriocins (substances that act as antibiotics), or the combination of several of these compounds (Gillor, Etzion, & Riley, 2008), which are characteristic substances of these microorganisms' metabolism, demonstrating the importance and interest in the use of these bacteria in food or pharmaceutical compounds for the purpose of becoming part of the human and animal microbiota.

**Table 6.** Hydrophobicity of the *Lactobacillus acidophilus* strains' cell surface by bacterial adhesion to hydrocarbons.

Origins	Adhesion		
	Xylol	Ethyl acet.	Chloroform
LA1	45.00 <sup>aA</sup> (±1.88)	72.52 <sup>bB</sup> (±3.01)	84.03 <sup>aB</sup> (±1.60)
LA2	55.75 <sup>aA</sup> (±1.25)	84.16 <sup>bA</sup> (±1.60)	98.41 <sup>aA</sup> (±0.90)
LA3	48.96 <sup>aA</sup> (±1.37)	77.34 <sup>bB</sup> (±1.89)	76.90 <sup>aC</sup> (±1.04)

\*Values are represented by mean ± standard deviation of three independent experiments. Equal lowercase letters in the same row do not differ from each other for the Tukey Test with  $p > 0.05$ . Equal uppercase letters in the same column do not differ from each other for Tukey's test with  $p > 0.05$ .

Using the same methodology, Pereira and Gómez (2007) reported inhibition of *E. coli* and *S. aureus* by *L. acidophilus* obtained from a commercial lyophilized probiotic culture.

Similar results were described by Costa, Suguimoto, Miglioranza, and Gómez (2012), who achieved inhibition of *E. coli* by culturing a strain of *L. acidophilus* through the multilayer methodology.

#### Bile salt hydrolase activity (BSH)

BSH is an enzyme produced by several microorganisms, including probiotics. Many studies involving the kinetics of this enzyme have reported its efficiency in hydrolyzing tauroconjugated bile salts and, because of this action, it has been demonstrated that this mechanism assists in the reduction of total blood cholesterol concentrations (Tanaka et al., 2000), which is why the production of this enzyme by probiotic bacteria has been widely studied.

For the medium containing GDCA there was no growth of LA strains.

The three strains of *Lactobacillus acidophilus* decongest bile salts present in a TDCA-containing medium. This effect was observed by the formation of white precipitation around the colonies in an MRS medium containing bile salts.

Evidence suggests that probiotics exert various biological health properties, one of which is the activity of anticholesterolemic bile salts through hydrolysis and cholesterol uptake (Nagpal et al., 2012). The selection of specific strains and the evidence of their efficacy results in a control of lipemic values that can be exploited to formulate new probiotic foods or supplements that, in turn, may play a role in the prevention of cardiovascular diseases.

The properties of lactobacilli, which influence cholesterol decrease, have been evaluated *in vivo* in several studies in humans and animal models, mostly consisting of the consumption of supplements and fermented foods containing selected *Lactobacillus* strains (Kumar, Batish, & Grover, 2011; Jones, Tomaro-Duchesneau, Martoni, & Prakash, 2013). Schillinger, Guigas, and Holzapfel (2005), in a study which isolated different *Lactobacillus* from commercial yogurts, found that all *L. acidophilus* strains produced precipitation zones in the BSH plaque trial.

#### Conclusion

The results obtained show that LA2 used in fermented foods was distinguished in relation to the other origins, since it presented a higher growth

rate, advantage in intestinal colonization, and an antibiotic resistance profile against clindamycin, gentamicin, and erythromycin, helping to restore intestinal flora during antibiotic therapy. It registered greater resistance to digestive and intestinal enzymes and adherence to the MATS test.

Thus, it is possible to have equal or better probiotic effect using products fermented with *Lactobacillus acidophilus* as compared to products purchased in compounding or dispensing pharmacies.

#### References

- Agência Nacional de Vigilância Sanitária [Anvisa]. (2016). *Alimentos com alegações de propriedades funcionais e ou de saúde, novos alimentos/ingredientes, substâncias bioativas e probióticos: lista de alegações de propriedade funcional aprovadas*. Atualizado em 22/12/2016. Retrieved on July 20, 2017 from [www.portal.anvisa.gov.br/alimentos/alegacoes](http://www.portal.anvisa.gov.br/alimentos/alegacoes)
- Anal, A. K., & Singh, K. (2007). Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. *Trends in Food Science & Technology*, 18(5), 240-251. Doi: 10.1016/j.tifs.2007.01.004
- Andreasen, A. S., Larsen, N., Pedersen-Skovsgaard, T., Berg R. M. G., Møller, K., Svendsen, K. D., ... Pedersen, B. K. (2010). Effects of *Lactobacillus acidophilus* NCFM on insulin sensitivity and the systemic inflammatory response in human subjects. *British Journal of Nutrition*, 104(12), 1831-1838. Doi: 10.1017/S0007114510002874
- Barreto, G. P. M., Silva, N., Silva, E. N., Botelho, L., Yim, D. K., Almeida, C. G., & Saba, G. L. (2003). Quantificação de *Lactobacillus acidophilus*, bifidobactérias e bactérias totais em produtos probióticos comercializados no Brasil. *Brazilian Journal of Food Technology*, 6(1), 119-126.
- Bertazzoni-Minelli, E., & Benini, A. (2008). Relationship between number of bacteria and their probiotic effects. *Microbiology and Ecology of Health and Disease*, 20(4), 180-183. Doi: 10.1080/08910600802408095
- Castillo, N. A., Moreno de LeBlanc, A. M., Galdeano, C., & Perdigón, G. (2013). Comparative study of the protective capacity against *Salmonella* infection between probiotic and nonprobiotic *Lactobacilli*. *Journal of Applied Microbiology*, 114(3), 861-876. Doi: 10.1111/jam.12074
- Charteris, W. P., Kelly, P. M., Morelli, L., & Collins, J. K. (1998). Antibiotic susceptibility of potentially probiotic *Lactobacillus* species. *Journal of Food Protection*, 61(12), 1636-1643. Doi: 10.4315/0362-028X-61.12.1636
- Collado, M. C., Isolauri, E., Salminen, S., & Sanz, Y. (2009). The impact of probiotic on gut health. Development and application of an *in vitro* methodology to determine the transit tolerance of potentially probiotic *Lactobacillus* and *Bifidobacterium* species in the upper human

- gastrointestinal tract. *Current Drug Metabolism*, 10(1), 68-78. Doi: 10.1046/j.1365-2672.1998.00407.x
- Cordonnier, C., Thévenot, J., Etienne-Mesmin, L., Denis, S., Alric, M., Livrelli, V., & Blanquet-Diot, S. (2015). Dynamic In Vitro Models of the Human Gastrointestinal Tract as Relevant Tools to Assess the Survival of Probiotic Strains and Their Interactions with Gut Microbiota. *Microorganisms* 3(4), 725-745. Doi: 10.3390/microorganisms3040725
- Costa, G. N., Suguimoto, H. H., Miglironza, L. H. S., & Gómez, R. J. H. C. (2012). Atividade antimicrobiana de *Lactobacillus* e *Bifidobacterium* frente a microrganismos patogênicos "in vitro". *Semina: Ciências Agrárias*, 33(5), 1839-1846. Doi: 10.5433/1679-0359.2012v33n5p1839
- Danielsen, M., & Wind, A. (2003). Susceptibility of *Lactobacillus* spp. to antimicrobial agents. *International Journal of Food Microbiology*, 82(1), 1-11. Doi: 10.1016/S0168-1605(02)00254-4
- Darsanaki, R. K., Aliabadi, M. A., & Chakoosari, M. M. D. (2013). Antibiotic resistance of lactic acid bacteria. *Scientific Journal of Microbiology*, 2(11), 201-206. Doi: 10.14196/sjm.v2i11.1051
- De Vrese, M., & Schrezenmeir, J. (2008). Probiotics, prebiotics, synbiotics. *Advances on Biochemistry Engineering and Biotechnology*, 111, 1-66. Doi: 10.1007/10\_2008\_097
- Diep, D. B., Håvarstein, L. S., & Nes, I. F. (1995). A bacteriocin-like peptide induces bacteriocin synthesis in *Lactobacillus plantarum* C11. *Molecular Microbiology*, 18(4), 631-639. Doi: 10.1111/j.1365-2958.1995.mmi\_18040631.x
- Giaouris, E., Chapot-Chartier, M. P., & Briandet, R. (2009). 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*, 131(1), 2-9. Doi: 10.1016/j.ijfoodmicro.2008.09.006
- Gillor, O., Etzion, A., & Riley, M. (2008). The dual role of bacteriocins as anti-and probiotics. *Applied Microbiology and Biotechnology*, 81(4), 591-606. Doi: 10.1007/s00253-008-1726-5.
- Gueimonde, M., Sánchez, B. G., de Los Reyes-Gavilán, C., & Margolles, A. (2013). Antibiotic resistance in probiotic bacteria. *Frontiers in Microbiology*, 18(4), 202. Doi: 10.3389/fmicb.2013.00202
- Jones, M. L., Tomaro-Duchesneau, C., Martoni, C. J., & Prakash, S. (2013). Cholesterol lowering with bile salt hydrolase-active probiotic bacteria, mechanism of action, clinical evidence, and future direction for health applications. *Expert Opinion on Therapeutic*, 13(5), 631-642. Doi: 10.1517/14712598.2013.758706
- Kumar, M., Nagpal, R., Kumar, R., Hemalatha, R., Verma, V., & Kumar, A. (2012). Cholesterol-lowering probiotics as potential biotherapeutics for metabolic diseases. *Experimental Diabetes Research*, 2012, 1-14. Doi: 10.1155/2012/902917
- Kumar, R., Batish, V. K., & Grover, S. (2011). Hypocholesterolaemic effect of dietary inclusion of two putative probiotic bile salt hydrolase-producing *Lactobacillus plantarum* strains in Sprague-Dawley rats. *British Journal of Nutrition*, 105(4), 561-573. Doi: 10.1017/S0007114510003740
- Larsen, N., Michaelsen, K., Pærregaard, A., Vogensen, T., & Jakobsen, M. (2009). A comparative study on adhesion and recovery of potential probiotic strains of *Lactobacillus* spp. by in vitro assay and analysis of human colon biopsies. *Microbial Ecology and Health Disease*, 21(2), 95-99. Doi: 10.1080/08910600902907632
- Lebeer, S., Vanderleyden, J., & De Keersmaecker, S. C. (2008). Genes and molecules of lactobacilli supporting probiotic action. *Microbiology and Molecular Biology Reviews*, 72(4), 728-764. Doi: 10.1128/MMBR.00017-08
- Madureira, A. R., Soares, J. C., Pintado, M. E., Gomes, A. M. P., Freitas, A. C., & Malcata, F. X. (2008). Sweet whey cheese matrices inoculated with the probiotic strain *Lactobacillus paracasei* LAFTI\_L26. *Dairy Science and Technology*, 88(6), 649-665. Doi: 10.1051/dst:2008026
- Mangoni, J., Pozza, M. S. S., Sabedot, M. A., Pozza, P. C., Almeida, S., & Heinzen, E. L. (2011). Potencial probiótico de lactobacilos de origem suína. *Acta Scientiarum. Animal Sciences*, 33(3), 267-272. Doi: 10.4025/actascianimsci.v33i3.9826
- Mattia, A., & Merker, R. (2008). Regulation of probiotic substances as ingredients in foods: premarket approval or "generally recognized as safe" notification. *Clinical Infection Disease*, 46(Suppl. 2), S115-S118. Doi: 10.1086/523329
- Nader-Macías, M. E. F., Otero, M. C., Espeche, M. C., & Maldonado, N. C. (2008). Advances in the design of probiotic products for the prevention of major diseases in dairy cattle. *Journal of Industrial Microbiology and Biotechnology*, 35(11), 1387-1395. Doi: 10.1007/s10295-008-0438-2
- Nagpal, R., Kumar, A., Kumar, M., Behare, P. V., Jain, S., & Yadav, H. (2012). Probiotics, their health benefits and applications for developing healthier foods: a review. *FEMS Microbiology Letters*, 334(1), 1-15. Doi: 10.1111/j.1574-6968.2012.02593.x
- National Committee For Clinical Laboratory Standards [NCCLS]. (2015). *Performance standards for antimicrobial disk susceptibility tests: approved standard* (Document M02-A12, 12th ed.). Wayne, MI: NCCLS.
- Park, Y. H., Kim, J. G., Shin, Y. W., Kim, S. H., & Whang, K. Y. (2007). Effect of dietary inclusion of *Lactobacillus acidophilus* ATCC 43121 on cholesterol metabolism in rats. *Journal of Microbiology and Biotechnology*, 17(4), 655-662.
- Park, Y. H., Kim, J. G., Shin, Y. W., Kim, H. S., Kim, Y. J., Chun, T., ... Whang, K. Y. (2008). Effects of *Lactobacillus acidophilus* 43121 and a mixture of *Lactobacillus casei* and *Bifidobacterium longum* on the serum cholesterol level and fecal sterol excretion

- in hypercholesterolemia induced pigs. *Bioscience, Biotechnology, and Biochemistry*, 72(2), 595-600.
- Pelletier, C., Bouley, C., Cayuela, C., Bouttier, S., Bourlioux, P., & Bellon-Fontaine, M. N. (1997). Cell surface characteristics of *Lactobacillus casei* subsp. *casei*, *Lactobacillus paracasei* subsp. *paracasei*, and *Lactobacillus rhamnosus* strains. *Applied and Environmental Microbiology*, 63(5), 1725-1731.
- Pereira, V. G., & Gómez, R. J. H. C. (2007). Atividade antimicrobiana de *Lactobacillus acidophilus*, contra microrganismos patogênicos veiculados por alimentos. *Semina: Ciências Agrárias*, 28(2), 229-240.
- Pithva, S., Shekh, S., Dave, J., & Vyas, B. R. (2014). Probiotic attributes of autochthonous *Lactobacillus rhamnosus* strains of human origin. *Applied Biochemistry and Biotechnology*, 173(1), 259-277. Doi: 10.1007/s12010-014-0839-9
- Rosenberg, M., Gutnick, D., & Rosenberg, E. (1980). Adherence of bacteria to hydrocarbons: a simple method for measuring cell-surface hydrophobicity. *FEMS Microbiology Letters*, 9(1), 29-33. Doi: 10.1111/j.1574-6968.1980.tb05599.x
- Sathyabama, S., Vijayabharathi, R., Bruntha, D. P., Ranjithkumar, M., & Brinda Priyadarisini, V. (2012). Screening for probiotic properties of strains isolated from feces of various human groups. *Journal of Microbiology*, 50(4), 603-612. Doi: 10.1007/s12275-012-2045-1
- Savard, P., Lamarche, B., Paradis, M. E., Thiboutot, H., Laurin, E., & Roy, D. (2011). Impact of *Bifidobacterium animalis* subsp. *lactis* BB-12 and *Lactobacillus acidophilus* LA-5 containing yoghurt, on fecal bacterial counts of healthy adults. *International Journal of Food Microbiology*, 149(1), 50-57. Doi: 10.1016/j.ijfoodmicro.2010.12.026
- Schillinger, U., Guigas, C., & Holzapfel, W. H. (2005). In vitro adherence and other properties of lactobacilli used in probiotic yoghurt-like products. *International Dairy Journal*, 15(12), 1289-1297. Doi: 10.1016/j.idairyj.2004.12.008
- Sinn, D. H., Song, J. H., Kim, H. J., Lee, J. H., Son, H. J., Chang, D. K., ... Rhee, P. L. (2008). Therapeutic effect of *Lactobacillus acidophilus*-SDC 2012, 2013 in patients with irritable bowel syndrome. *Digestive Diseases and Sciences*, 53(10), 2714-2718. Doi: 10.1007/s10620-007-0196-4.
- Stefe, C. A., Alves, M. A. R., & Ribeiro, R. L. (2008). Probióticos, Prebióticos e Simbióticos – Artigo de Revisão. *Revista Saúde e Ambiente*, 1(3), 16-33.
- Tanaka, H., Hashiba, H., Kok, J., & Mierau, I. (2000). Bile salt hydrolase of *Bifidobacterium longum*: biochemical and genetic characterization. *Appl. Environ. Microbiol.*, 66(6), 2502-2512. Doi: 10.1128/AEM.66.6.2502-2512.2000
- Thumu, S. C., & Halami, P. M. (2012). Acquired resistance to macrolide-lincosamide-streptogramin antibiotics in lactic Acid bacteria of food origin. *Indian Journal of Microbiology*, 52(4), 530-537. Doi: 10.1007/s12088-012-0296-5
- Vizoso Pinto, M. G., Franz, C. M. A. P., Schillinger, U., & Holzapfel, W. H. (2006). *Lactobacillus* spp., with in vitro probiotic properties from human faeces and traditional fermented products. *International Journal of Food Microbiology*, 109(3), 205-214. Doi: 10.1016/j.ijfoodmicro.2006.01.029
- Zayed, G., & Winter, J. 1995. Batch and continuous production of lactic acid from salt whey using free and immobilized cultures of *Lactobacilli*. *Applied Microbiology and Biotechnology*, 44(3-4), 362-366. Doi: 10.1007/BF00169930

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