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In Vitro evaluation of probiotic properties of lactic acid bacteria strains isolated from juçara fruit native to the Atlantic Forest

Avaliação de cepas de bactérias ácido láticas isoladas de frutos juçara nativos da Mata Atlântica quanto a propriedades probióticas *In Vitro*

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Highlights

Identification of new probiotic candidates from natural sources.
Lactic acid bacteria isolated from juçara palm fruits.
Potential probiotic characteristics *in vitro* of native lactic acid bacteria.
Tolerance to simulated gastrointestinal conditions of lactic acid bacteria.

Abstract

Lactic acid bacteria (LAB) are the microorganisms most commonly used as probiotics. Since probiotic benefits are strain-dependent, there is a continuous need for research into new cultures with probiotic properties. Fruits such as juçara (*Euterpe edulis* Martius), a palm species from the Atlantic Forest threatened with extinction, are rich niches for microorganisms, including LAB. This study investigated the probiotic properties of *Lactococcus lactis* J7 and *Leuconostoc pseudomesenteroides* JF17 strains isolated from juçara fruits native to the Atlantic Forest. Probiotic characteristics, such as tolerance to simulated gastrointestinal fluids or juices, hydrophobicity, autoaggregation, coaggregation properties, inhibition of pathogenic microorganisms, and technological properties were evaluated. The survival rate

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of *L. lactis* J7 and *L. pseudomesenteroides* JF17 decreased after exposure to simulated gastrointestinal conditions; however, *L. lactis* J7 was more resistant, maintaining viability at the end of the enteric phase of 6.07 ± 0.16 log CFU mL^{-1}. The J7 strain also exhibited the highest values in hydrophobicity (12.55 ± 0.52%), autoaggregation (25.63 ± 0.75%), and coaggregation capacity with *E. coli* ATCC 25922 (21.52 ± 0.98%) and *S.* Enteritidis ATCC 13076 (22.68 ± 1.01%). Both J7 and JF17 strains demonstrated antimicrobial activity, confirmed by the multilayer agar plate system. Additionally, the growth of the strains was temperature-dependent, and they were able to thrive in high concentrations of sodium chloride (6%). Thus, *L. lactis* J7 is a promising probiotic candidate for the development of functional products. Nevertheless, further studies using animal models are necessary to explore the properties of these probiotic bacteria. Novel strains isolated from fruits should be studied to broaden the application of probiotic microorganisms in the development of foods and medicines.
Key words: *Lactococcus lactis. Leuconostoc pseudomesenteroides. Euterpe edulis* Martius. Aggregation activity. Simulated gastrointestinal.

Resumo

As bactérias ácido lácticas (BAL) são os microrganismos mais comuns aplicados como probióticos. Os benefícios dos probióticos dependem do tipo de cepa utilizada, portanto há sempre a necessidade de investigar novas culturas com propriedades probióticas. Frutas como a juçara, palmeira da Mata Atlântica, ameaçada de extinção, são nichos ricos em microrganismos, inclusive BAL. Assim, neste estudo foram investigadas as propriedades probióticas das cepas *Lactococcus lactis* J7 e *Leuconostoc pseudomesenteroides* JF17, isoladas de frutos de juçara (*Euterpe edulis* Martius), nativa da Mata Atlântica. Foram avaliadas características probióticas, como tolerância a sistemas gastrointestinais simulados, hidrofobicidade, propriedades de autoagregação e coagregação, inibição de microrganismos patogênicos e suas propriedades tecnológicas. A taxa de sobrevivência de *L. lactis* J7 e *L. pseudomesenteroides* JF17 diminuiu após condições gastrointestinais simuladas, porém, *L. lactis* J7 foi mais resistente, mantendo uma viabilidade ao final da fase entérica de 6,07 ± 0,16 log UFC mL^{-1}. A cepa J7 também apresentou o maior valor em termos de hidrofobicidade (12,55 ± 0,52%), autoagregação (25,63 ± 0,75%) e capacidade de coagregação com *E. coli* ATCC 25922 (21,52 ± 0,98%) e *S.* Enteritidis ATCC 13076 (22,68 ± 1,01%). No entanto, tanto J7 quanto JF17 tiveram atividade antimicrobiana confirmadas pelo sistema de placas de ágar multicamadas. Além disso, o crescimento das cepas foi influenciado pela temperatura, e elas conseguiram crescer em altas concentrações de cloreto de sódio (6%). Assim, *L. lactis* J7 é um candidato probiótico promissor para o desenvolvimento de produtos funcionais. No entanto, mais estudos devem ser realizados para investigar as propriedades destas bactérias probióticas utilizando modelos animais. Novas cepas isoladas de frutos devem ser estudadas para ampliar o uso de microrganismos probióticos no desenvolvimento de alimentos e medicamentos.
Palavras-chave: *Lactococcus lactis. Leuconostoc pseudomesenteroides. Euterpe edulis* Martius. Atividade de agregação. Sistema gastrointestinal simulado.

Introduction

Lactic acid bacteria (LAB), widespread in nature, are among the most important microorganisms primarily known for producing fermented foods (Grujović et al., 2022). Additionally, LAB are the most commonly applied microorganisms as probiotics. They are desirable members of the gastrointestinal tract microbiota and possess Generally Recognized As Safe (GRAS) status (Linares et al., 2017). According to the Food and Agriculture Organization of the United Nations / World Health Organization [FAO / WHO] (2002), probiotics are living microorganisms that, when administered in adequate amounts, confer health benefits to consumers. Studies have linked dysbiosis of the intestinal microbiota to various pathological conditions, including respiratory diseases. There is substantial scientific evidence supporting the ability of probiotics to enhance human immunity at recommended doses. This enhancement can help prevent colonization by pathogenic microorganisms and reduce the incidence and severity of foodborne infections, as well as infections caused by agents such as Covid-19 (Olaimat et al., 2020; Mendonça et al., 2023).

The beneficial effects of probiotics are strain-specific, highlighting the importance of exploring various natural sources to identify new probiotic candidates (Sionek et al., 2023). While most probiotics used in commercial products are from the genera *Lactobacillus* and *Bifidobacterium,* they are not the only sources (Sharma et al., 2021). Numerous LAB strains isolated from different food sources have been studied for their probiotic potential (Hwanhlem et al., 2017; Nami et al., 2019; Topçu et al., 2020; Xu et al., 2020). To assess the probiotic properties of microorganisms, *in vitro* assays are routinely used as an alternative to more complex and costly *in vivo* experiments (Huang et al., 2017).

In vitro tests, developed as selection criteria for potential probiotics, require that a probiotic strain survive the low pH of the intestinal tract and stomach and tolerate the damaging effects of gastrointestinal enzymes (Makinen et al., 2012). Potential probiotics must adhere to and colonize the intestinal cell wall to trigger direct interactions with host cells, leading to the competitive exclusion of pathogens and modulation of host responses (Ouwehand et al., 2001). Furthermore, when intentionally added to food, they must remain viable during product manufacturing and storage (Palanivelu et al., 2022).

Therefore, *in vitro* models of gastrointestinal digestion, adhesion, and autoaggregation assays are crucial in testing the applicability of probiotic strains. However, maintaining high viability of free probiotic bacteria in human and animal organisms presents a challenge due to adverse conditions in the gastrointestinal tract, such as the presence of acid, bile, and digestive enzymes (Huang et al., 2017). Moreover, obtaining genetically stable strains for use in probiotic foods has been recognized as an important factor (Palanivelu et al., 2022). Food processing techniques, such as the use of high temperatures, high concentrations of salts, and pH adjustments, can damage beneficial bacteria, leading to decreased viability. Hence, screening and selecting new probiotic strains with greater viability is essential (Cassani et al., 2020).

Fruits and vegetables host a rich autochthonous microbiota, including LAB strains with yet-to-be-discovered probiotic properties that could be harnessed to develop novel health-promoting fermented foods (Sakandar et al., 2019). Brazil, with its vast biodiversity, features the Atlantic Forest, which is renowned for its variety of fruits. Owing to its rich biodiversity, certain fruits, such as juçara palm (*Euterpe edulis* Martius), are notable for their nutritional value. Known primarily for the extraction of its heart of palm, which has placed it on the list of species threatened with extinction, the sustainable exploitation of its fruits has contributed to its preservation (Miranda et al., 2020).

To the best of our knowledge, no research has yet evaluated the use of autochthonous LAB from juçara palm fruits. Investigating novel strains is essential to expand the application of probiotic microorganisms in food production. Therefore, the aim of this study is to identify new probiotic candidates from LAB isolated from juçara fruits and to explore the potential probiotic properties of these cultures.

Material and Methods

Isolation of LAB from juçara fruit

The LAB strains were isolated from samples of the juçara fruit, harvested in May of the years 2017 and 2018, from Bimini Farm (23º14"0.48'S; 51º 4" 0.43' W), in the region of Rolandia, Brazil. Juçara fruit was protected by genetic heritage with the A701C5B registration. The fruits (1 kg) were processed immediately after collection, washed, and pulped (1: 1 / fruits: sterile water) in a planetary mixer (Arno, 408002194 BPA, Brazil) previously sanitized. The pulp obtained was fractionated in erlenmeyer flasks (n = 5) and incubated at 25 °C for 48 h (Incubator Novatecnica®, Brazil). After this period, serial dilutions of fermented juçara pulp were prepared in 0.1% (w v^{-1}) peptone water followed by plating these dilutions in MRS (Merck®, Germany) agar plates. These plates were incubated at 30 °C for 48 h for the isolation of LAB species. Following the cultivation of LAB, colonies were randomly selected from corresponding agar plates and tested for cell morphology, Gram stain and catalase reaction (Öz et al., 2017). In total, 198 isolates were subsequently selected for their morphological and molecular characterization of the16S rRNA.

Identification of LAB through 16s rRNA sequencing

Seven identical colonies with circular and smooth structure, cocci or rod morphology, Gram positive, catalase negative, and that ferment glucose with gas production, were subjected to molecular characterization to sequencing of the 16S rRNA molecular marker. For the identification of LAB by 16S rRNA sequencing, the MiSeq Sequencing System (Illumina Inc., USA) and Next Generation Sequencing (NGS) (Neoprospecta Microbiome Technologies, Florianopolis, Brazil) were used. The amplicon sequencing library preparation was performed for bacteria using the VS - V4 16S rRNA gene primers 341 F (CCTACGGGRSGCAGCAG; 5' - 3' (Y. Wang & Quian, 2009) and 806 R (GGACTACHVGGGTWTCTAAT; 5' - 3' (Caporaso et al., 2011). The identified LAB was registered with GenBank and were stored in MRS broth supplemented with 20%

(w v^{-1}) glycerol at – 80 °C. Microorganisms were also protected by Brazilian genetic heritage with the A02124C and A38EECB registers to *L. pseudomesenteroides* and *L. lactis,* respectively.

Potential probiotic characteristics in vitro

Tolerance to simulated gastrointestinal (GIT) conditions

The tolerance to simulated GIT conditions test was performed by successively exposing the strains to gastric and enteric simulated juices as described by Bedani et al. (2014). *L. lactis* J7 and *L. pseudomesenteroides* JF17 were grown for 24 h at 30 °C in MRS broth (~ 9 log CFU mL^{-1}). Ten milliliters from each triplicate dilution of culture of strains J7 and JF17 in 0.85 g 100 mL^{-1} NaCl (Synth, Brazil) solution were transferred to 3 sterile flasks, with a total of 9 flasks containing the samples, and pH was adjusted to 2.0 - 2.5 with HCl 1 N (Synth, Brazil). In addition, pepsin (from porcine gastric mucosa, Sigma-Aldrich) and lipase (from *Rhizopus oryzae,* Sigma-Aldrich) were added to samples reaching a final concentration of 3 g L^{-1} and 0.9 mg L^{-1}, respectively. Flasks were incubated at 37 °C for 2 h in a shaker with agitation of 150 rpm (NT233, Novatecnica, Brazil), leading to the simulated gastric phase. Then, the pH of samples was adjusted to 4.5 – 5.5 using a pH 12 buffer solution (Hexis®). Bile (bovine bile, Sigma-Aldrich) and pancreatin (pancreatin from porcine pancreas, Sigma-Aldrich) were added to a final concentration of 10 g L^{-1} and of 1 g L^{-1}, respectively. After, samples were incubated again at 37 °C for 2 h under agitation to simulate enteric phase I. Finally, the pH was adjusted to 6.5 – 7.5 using the same buffer solution (pH 12), containing bile and pancreatin adjusted to maintain the concentration of 10 g L^{-1} and 1 g L^{-1}, respectively, and the samples were incubated again at 37 °C for 2 h under agitation to simulate enteric phase 2 and reaching 6 h of assay.

Viable counts of strains were determined by plate method using MRS agar after serial dilution in maximum recovery. MRS agar plates were incubated anaerobically at 37 °C for 48 h and the colony forming units estimated. Survival rate was calculated according to the following Equation (1),

$$\text{Survival rate (\%)} = \log \text{CFU } N_1 / \log \text{CFU } N_0 \times 100 \quad (1)$$

N_1, the total viable count of probiotic strains after exposure to *in vitro* GTI conditions (6 h); N_0, the total viable count of probiotic strains before exposure to *in vitro* GTI conditions (0 h).

Autoaggregation assays

Autoaggregation assays were carried out according to Collado et al. (2008) using the autoaggregation percentage. J7 and JF17 strains were grown at 30 °C for 20 h in MRS broth. The bacteria cells were harvested by centrifugation at 10,000 × g for 10 min at 4 °C and washed twice with phosphate-buffered saline (PBS) pH 7.2, and then resuspended in the PBS. Absorbance at a wavelength of 600 nm (A_{600} nm) was adjusted to 0.25 ± 0.05 to standardize the number of bacteria (7 – 8 log CFU mL^{-1}). Then, the bacterial suspensions were incubated in 1 mL aliquots at 37 °C and monitored at different time intervals (0, 2, 4, 6, 10 and 20 h). The absorbance was recorded and the percentage of autoaggregation

(AA%) was calculated according to the Equation (2):

$$AA\ (\%) = [\ 1 - A_t / A_0] \times 100 \quad (2)$$

where A_0 represents the absorbance (A_{600} nm) at 0 h and A_t represents the absorbance (A_{600} nm) at different time intervals (2, 4, 6, 10 and 20 h).

Coaggregation assays

Bacterial suspensions were prepared as described for autoaggregation analysis. Equal volumes of cells (1 mL) of the different probiotics and strains (*Escherichia coli* ATCC 25922 and *Salmonella enterica* serovar Enteritidis ATCC 13076) were mixed and incubated at 37 °C without agitation. The A_{600} nm of the mixtures described above was monitored at different times (0, 2, 4, 6, 10 and 20 h). Absorbance was determined for the mixture and for the bacterial suspensions alone (Collado et al., 2008). Coaggregation was calculated as follows Equation (3),

$$AC = \{[(A_{pat} + A_{probio}) / 2 - A_{mix}] / (A_{pat} + A_{probio}) / 2\} \times 100 \quad (3)$$

where A_{pat} and A_{probio} represent A_{600} nm of the separate bacterial suspensions (pathogen and probiotic strains respectively) in control tubes and A_{mix} represents the absorbance of the mixed bacterial suspension at different time intervals (2, 4, 6, 10 and 20 h).

Cell surface hydrophobicity

According to Tallapragada et al. (2018), 24 h old bacterial suspensions were prepared as described for autoaggregation analysis and the pellet was washed with sterile saline solution (pH 6) and re-suspended in the same solution and OD was measured at 580 nm. The suspension (1.5 mL) was mixed with n-hexadecane in 1: 1 (v v^{-1}) ratio and vortexed for 2 min. Two phases separated after 30 min of incubation and OD was measured at 580 nm. The percentage of hydrophobicity was measured according to Equation (4).

$$\text{Hydrophobicity}\ (\%) = [(OD_0 - OD_{30}) / OD_0] \times 100 \quad (4)$$

where OD_0 and OD_{30} refer to the initial OD and OD measured after 30 min, respectively.

Inhibition of pathogens by multilayer agar plate system

The method of inhibition by the multilayer agar plate system was described by Diep et al. (1995), with some modifications. The following layers of agar were added to each plate for testing, 7 ml of MRS agar (1.4% agar) without microorganisms; 7 mL of MRS agar (0.7% agar) plus 0.5 mL of the activated culture of *L. lactis* J7 or *L. pseudomesenteroides* JF17, previously diluted in 0.1% peptone water, to approximately 3 log CFU mL^{-1}; 7 mL of BHI agar (Merck®) (0.7% agar) without microorganisms. After incubation at 37 ºC for 72 h, for growth of lactic acid bacteria, the last layer was added, containing 7 mL of BHI agar added with 50 µL of a 24 h culture (*E. coli* ATCC 25922 or *S. Enteritidis* ATCC 13076), previously diluted in 0.1% peptone water, up to approximately 5 log CFU mL^{-1}. The plates were incubated at 37 ºC for another 24 h. In case of inhibition, there were halos of non-development of the indicator pathogenic microorganism around the potentially probiotic colonies.

Technological properties of the probiotic strains

Optimum temperature and growth tolerance in the presence of sodium chloride was tested as described by Benavides et al. (2016). *L. lactis* J7 or *L. pseudomesenteroides* JF17 (7 log CFU mL^{-1}) was inoculated in tubes containing MRS and incubated at 15 °C and 45 °C for 24 h. To evaluate the tolerance in the presence of sodium chloride, the strains evaluated were inoculated in MRS containing 2, 4, and 6% NaCl for 24 h and incubation at 15 °C and 45 °C. The viability was monitored for each treatment and the effect of sodium chloride on cell survival was determined using the plate agar method. *Lactobacillus acidophilus* LA-5 was used as a probiotic control strain in this experiment. Not modified MRS was used as control and the experiment was run in triplicate starting from individual batches of bacterial culture.

Statistical analysis

The means were calculated from repeated measurements performed three times. The statistical analysis was carried out by one-way analysis of variance (ANOVA), and Tukey's post hoc test was used to determine significant differences between the means. The statistical significance considered was $p < 0.05$ (Statistica software 10.0).

Results and Discussion

Isolation of lactic acid bacteria

From the samples, seven predominant types of colonies were isolated. Morphologically the colonies were circular or oval, slightly raised, smooth structure, with an entire margin and creamy. Microscopically the strains were identified as Gram-positive, spherical-shaped. Biochemically strains showed catalase-negative reaction and positive for glucose fermentation. Two predominant colonies were identified by 16S rRNA sequencing method. The cultures were 100% like *Lactococcus lactis* and *Leuconostoc pseudomesenteroides.* Thus, the strains were named *L. lactis* J7 (Genbank-MW132602) and *L. pseudomesenteroides* JF17 (GenBank-MN756802).

Tolerance to simulated GIT conditions

The ability of *L. lactis* J7 and *L. pseudomesenteroides* JF17 strains, isolated from juçara fruits, to survive the simulated gastrointestinal conditions was evaluated. Figure 1 shows the results obtained. Thus, three relevant factors were considered, the influence of acid pH values with pepsin (gastric stress) and the additional action of bile salts and pancreatin (intestinal stress), simulating the successive gastric distribution of bacteria to the intestine during digestion.

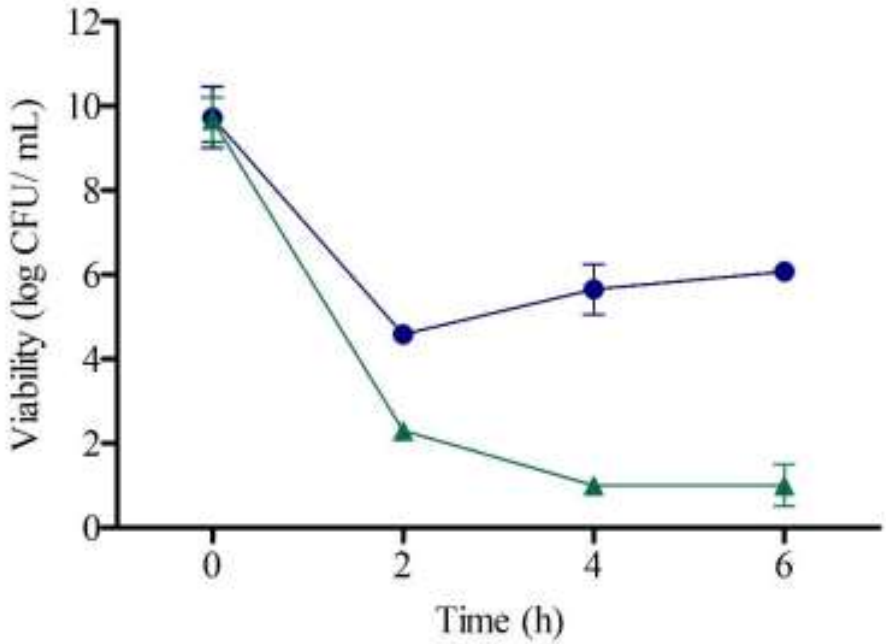

Figure 1. Survival of the isolates, *L. lactis* J7 and *L. pseudomesenteroides* JF17 (log CFU mL^{-1}), before (0 h) and during exposure to *in vitro* simulated gastric (2 h) and enteric (4 and 6 h) conditions (n = 3, x ± SD).

The simulated gastric passage (after 2 h of the *in vitro* test) was a critical step, which significantly decreased viability ($p < 0.05$) in approximately 5 and 7 logarithmic units, for J7 and JF17, respectively. After the complete simulation (6 h of incubation in enteric phase), the viability results were 6.07 ± 0.16 log CFU mL^{-1} for J7 and 1.05 ± 0.05 log CFU mL^{-1} for JF17. Thus, among the two strains studied, *L. lactis* J7 was the most resistant to simulated gastric and intestinal fluids, while *L. pseudomesenteroides* JF17 was sensitive to this treatment, showing a decrease in viability of almost 8 log CFU mL^{-1} (Figure 1).

Probiotics cannot affect the intestinal environment when ingested unless the population in the intestine reaches a minimum level of 6 to 8 log CFU g^{-1} of feces (Marteau & Rambaud, 1993), therefore, *L. lactis* J7 could positively influence the intestine of the host. According to Benavides et al. (2016) after incubation of the LAB strains analyzed under acidic conditions (pH 2.5) and biliary conditions (0.3%), all isolates, including *L. lactis* subsp. *lactis* UTNFa40, UTNFa38 and UTNFa41, were highly tolerant to acidic conditions (3 h of incubation) and resistant to bile (90% resistance), and after 4 h of incubation, there was a significant increase in viability (~ 9 - 9.5 log CFU mL^{-1}), as well as that observed in this work (Figure 1).

Although a log-scale reduction of viability may occur, the exposure to acid does not mean that the potential health benefits are lost. However, cells injured may recover

in the host's gastrointestinal tract, ensuring beneficial effects on health. The benefit also may be mediated, for example, through the components of the cell wall of the probiotics which will then be available in both dead and living cells (Almada et al., 2016).

It is relevant to highlight that *in vitro* tests are valuable for the selection of microorganisms for probiotic applications, such as resistance to low pH and high concentrations of bile salts, which are important for the growth and survival of bacteria in the intestinal tract (Todorov et al., 2011). Since bile salts disrupt the structure of the cell membrane, they are toxic to living cells. Therefore, bile tolerance is considered an important characteristic of the potential probiotic that enables it to survive, grow, and exert its action in gastrointestinal transit (Mendonça et al., 2023).

Autoaggregation and coaggregation activity

The results of autoaggregation assays are shown in Table 1. The highest autoaggregation was observed in *L. lactis* J7, whose autoaggregation percentage increased ($p < 0.05$) from 3.17 ± 0.05% to 25.63 ± 0.75% during 2 - 20 h. The studied strain of *L. pseudomesenteroides* JF17 also showed the ability to autoaggregation over time, however, its autoaggregation was approximately 2.3 times less than that presented by the J7 strain, at 20 h. This result may indicate that *L. lactis* J7 has a high potential to adhere to epithelial cells and mucous surfaces, and *L. pseudomesenteroides* JF17 has a lower capacity in this adhesion process (Table 1). Strains with less than 10% are considered to have weak autoaggregation (C. Y. Wang et al., 2010).

Table 1
Autoaggregation percentage of *L. lactis* J7 and *L. pseudomesenteroides* JF17

Strains	Time				
	2 h	4 h	6 h	10h	20 h
L. lactis J7	$3.17^{a} \pm 0.05$	$3.30^{a} \pm 0.31$	$6.70^{a} \pm 0.28$	$11.21^{a} \pm 1.44$	$25.63^{a} \pm 0.75$
L. pseudomesenteroides JF17	$2.94^{b} \pm 0.11$	$3.19^{a} \pm 0.16$	$5.22^{b} \pm 0.95$	$7.06^{b} \pm 0.73$	$11.16^{b} \pm 1.01$

The values shown are averages of triplicate determinations (n = 3, x ± SD). Same lowercase letters in the same column, are significantly equals ($p < 0.05$).

Compared with other LAB strains, autoaggregation ability of the isolates are within what has already been reported. The percentage of autoaggregation of *Bifidobacterium animalis* subsp *lactis* BB12 and *Lactobacillus casei* Shirota presented 36.7% and 17.9%, respectively (Yong et al., 2010). *L. lactis* LMG 7930 presented a percentage of 24.12% (Armas et al., 2017) and *L. lactis* KC24, 36.15% (Lee et al., 2015). There are not many reports describing molecules involved in the aggregation of *Leuconostoc* strains, however, Zhang et al. (2013) reported a 23.29% autoaggregation for *Leuconostoc lactis* isolated from intestine of black porgy fish.

The ability to adhere to epithelial cells and mucosal surfaces has been suggested to be an important property for probiotics (Bao et al., 2010). The autoaggregation property of the probiotic candidates is important since it is related to the type and the amount of surface layer protein that contributes to the bacterial adhesion onto the intestinal cell wall. A prerequisite attribute in providing beneficial health effects to the host (Ng et al., 2015; Tuo et al., 2013).

Bacterial aggregation between microorganisms of the same strain, autoaggregation, or between genetically different strains, coaggregation, is of considerable importance in several ecological niches, especially in the human gut. Therefore, the strains J7 and JF17 were not only examined for their autoaggregation ability, but also tested to determine the coaggregation with pathogenic bacteria.

Both strains, J7 and JF17, showed some coaggregation properties with *E. coli* ATCC 25922 and *S.* Enteritidis ATCC 13076 (Table 2). However, the coaggregation percentages depended on the specific strain (and strains of the pathogen) and on the incubation time. The *L. lactis* J7 strain showed the highest ($p < 0.05$) coaggregation capacity (22.68 ± 1.01%) with *S.* Enteritidis ATCC 13076, followed by 21.52 ± 0.98% with *E. coli* ATCC 25922. The percentage of *L. pseudomesenteroides* JF17 coaggregation was approximately 2.0 times lower for both pathogens, as also reported for autoaggregation.

Table 2
Coaggregation percentage with the *L. lactis* J7 and *L. pseudomesenteroides* JF17 strains against pathogens, *E. coli* ATCC 25922 and *S.* Enteritidis ATCC 13076

Time	*E. coli* ATCC 25922		*S.* Enteritidis ATCC 13076	
	L. lactis J7	*L. pseudomesenteroides* JF17	*L. lactis* J7	*L. pseudomesenteroides* JF17
2 h	2.90 ^{a} ± 0.36	2.97 ^{a} ± 0.15	3.08 ^{a} ± 0.55	3.12 ^{a} ± 0.48
4 h	4.06 ^{a} ± 0.24	3.98 ^{b} ± 0.45	3.35 ^{a} ± 0.65	6.15 ^{b} ± 0.55
6 h	5.33 ^{a} ± 0.58	5.15 ^{a} ± 0.58	6.64 ^{a} ± 0.22	8.52 ^{b} ± 0.89
10 h	10.25 ^{a} ± 0.99	6.89 ^{b} ± 0.22	12.58 ^{a} ± 0.88	9.31 ^{b} ± 0.88
20 h	21.52 ^{a} ± 0.98	9.46 ^{b} ± 0.84	22.68 ^{a} ± 1.01	12.87 ^{b} ± 1.02

The values shown are averages of triplicate determinations (n = 3, x ± SD). Same lowercase letters in the same line for the same pathogenic microorganism, are significantly equals ($p < 0.05$).

Coaggregation may play an important role in eliminating pathogens from the gastrointestinal tract. Coaggregation with a potential pathogen allows the probiotic strain to produce antimicrobial substances near them, which may inhibit the growth of pathogenic strains in the gastrointestinal tract (Tuo et al., 2013). Thus, the highest value for coaggregation and autoaggregation skills for *L. lactis* J7 suggests that this strain may help prevent colonization of the intestinal system by pathogens.

Cell surface hydrophobicity

For the hydrophobicity of the bacterial cell surface, *L. lactis* J7 was more adherent (12.55 ± 0.52%) to n-hexadecane than *L. pseudomesenteroides* JF17 (4.87 ± 0.15 %). The percentages of adherence of these strains tested seem to be lower than the *Lactobacillus* strains previously reported by Tuo et al. (2013), which varied between 26.71% (*L. plantarum* 130) and 89.97% (*L. casei* 137). However, they seem to be close to that reported by Tallapragada et al. (2018) for *Lactobacillus* sp. G3_4_1TO2 and *Lactobacillus fermentum,* which had hydrophobicity between 10 - 15%.

LAB strains with more than 40% affinity for polar solvents are generally more hydrophobic (Giaouris et al., 2009). Thus, in this study, none of the strains can be considered highly hydrophobic, but J7 strain was the one with the greatest adhesion capacity. As reported by Kimoto-Nira et al. (2010), the type of carbohydrate in the broth can affect the hydrophobicity of *Lactococcus lactis* G50 strain. According to the authors, hydrophobicity was 0.70% for fructose, 2.80% for sucrose, 4.20% for glucose, 11.7% for galactose, 14.1% for xylose and 46.8% for lactose. Other reports refer to the presence of the hydrophobicity property, the bacterial cells strongly adhere to the mucous cells of the host's intestine and exercise physiological functions (Rodrigues, 2011).

Inhibition of pathogens

It was found that the indicator strains, E. coli ATCC 25922 and S. Enteritidis ATCC 13076, showed sensitivity to the inhibition zone of *L. lactis* J7 and *L. pseudomesenteroides* JF17 when tested by multilayer agar plate system. Both *E. coli* and *Salmonella* are Gram-negative bacteria with a thinner layer of peptidoglycan in the cell wall. The relative efficacy of lactic acid against Gram-negative bacteria is not unexpected because this small water-soluble molecule gains access to the periplasm through the water-filled porin proteins of the outer membrane (Nikaido, 1996). The antimicrobial effect of organic acids lies in the reduction of pH, as well as the undissociated form of the molecules. It has been proposed that the low external pH causes acidification of the cell cytoplasm, while the undissociated acid can diffuse across the membrane (Ammor et al., 2006). Hwanhlem et al. (2017), found that all indicator strains showed sensitivity to the inhibition zone of *L. lactis* subsp. *lactis* KT2W2L when tested by the agar stain test. Y. Wang et al. (2018) showed that *Lactobacillus johnsonii* LY1 had high antibacterial efficacy against *E. coli* and *S.* Enteritidis, while *L. pseudomesenteroides* P1 had the most powerful capacity against *S. aureus.* Many of these authors also attribute the inhibitory effect to the production of organic acids, as

well as the synthesis of other compounds, such as hydrogen peroxide or bacteriocins, substances characteristic of the metabolism of these microorganisms (Costa et al., 2012).

Technological properties of the probiotic strains - Ideal growth temperature and sodium chloride tolerance

The LAB isolated from juçara fruits, *L. lactis* J7 and *L. pseudomesenteroides* JF17 grew at temperatures of 15 °C and 45 °C, in addition to exhibiting tolerance to sodium chloride (Figure. 2). At 15 °C and treatment with 2%, 4% and 6% NaCl, no significant difference was recorded for *L. lactis* J7. *L. pseudomesenteroides* JF17, showed stability at 2% and 4%, but not at 6%, when a decrease ($p < 0.05$) in viability was observed (7.49 ± 0.18 log CFU mL^{-1}). The control probiotic strain *Lactobacillus acidophilus* LA-5 remained viable unchanged at the tested NaCl concentrations, to 15 °C. (Figure 2a). The rapid change in the osmolarity, by adding NaCl, of the environment might compromise their essential functions before the bacteria can adapt to the new environment (Sunny-Roberts & Knorr, 2008).

Decreased viability was detected with 6% NaCl at both temperatures tested, although this drop was statistically significant and evident at 45 °C for both strains and the control ($p < 0.05$). At 45 °C a decrease in viability for J7 (9.08 ± 0.10 to 6.79 ± 0.05 log CFU mL^{-1}) and JF17 (5.35 ± 0.10 to 4.23 ± 0.34 log CFU mL^{-1}) when the NaCl concentration was increased from 2% to 6%, was observed (Figure 2b). The results also suggested that the growth of the strains was influenced by temperature, instead of by the concentration of NaCl added to the medium. The strains had the ability to grow in high concentrations of sodium chloride (6%), but at a low temperature of 15 °C. The same did not happen for 45 °C. In Fig. 2b it was also observed that the strain *L. pseudomesenteroides* JF17 did not have the ability to grow at a temperature of 45 °C, even when the NaCl concentration was low (2%), and its preference for low temperatures (15 °C) was evident (Figure 2a). The heat tolerance of LAB indicates their potential applications in some food manufacturing processes and formulation technologies under industrial conditions (Sánchez et al., 2012).

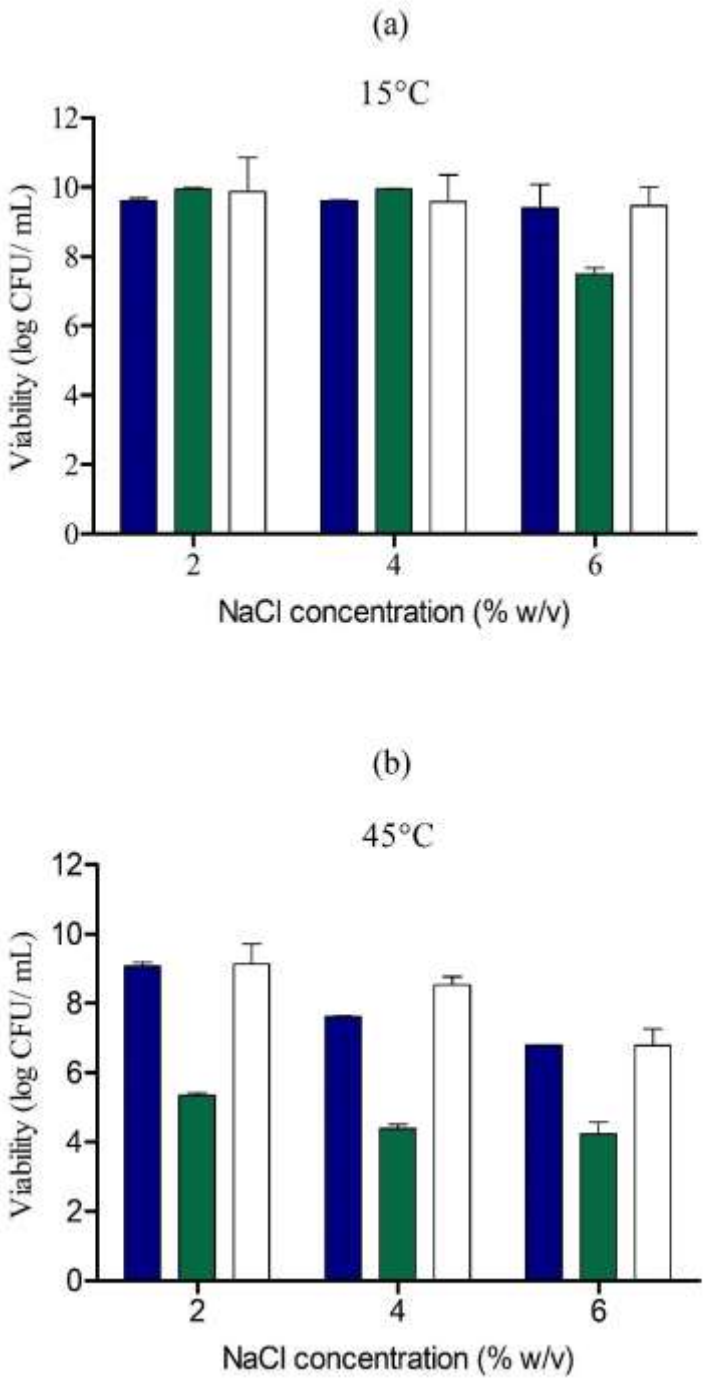

Figure 2. The effect of temperature and NaCl on the growth of ■ *L. lactis* J7 and ■ *L. pseudomesenteroides* JF17. (a) Viability at 15 °C and 2%, 4% and 6% NaCl. (b) Viability at 45 °C and 2%, 4% and 6% NaCl. Bars are means ± standard error of the mean. Control: ▭ *Lactobacillus acidophilus* LA-5®.

According to Ng et al. (2015) isolates that grow to a concentration of 4% NaCl can be considered as having the main characteristics of a starter culture strain, without support for higher levels of sodium chloride. Smith et al. (2010) while studying the molecular mechanisms of stress resistance in *Lactococcus lactis* stated that at salt concentrations of 3 to 5% w v^{-1} NaCl, the culture produces more lactic acid, but its growth is inhibited at 6% w v^{-1} NaCl. In many products the addition of salt is necessary, as a preservative, to give a pleasant taste or even as a proteolysis enhancer, as in fermented dairy products, especially cheeses (Gonzalez & Aryana, 2018). Cheese is an advantageous probiotic carrier due to its consistency and versatility compared to fermented milk. It has the highest consumption among dairy products globally and is proposed as an excellent alternative for delivering probiotic bacteria (Meidistria et al., 2020).

Thus, tolerance to the harsh conditions during food production very often constitutes a critical point for the inclusion of LAB into food products. Selection of salt tolerant strains can open a new array of opportunities for the development of novel products with LAB strain and probiotic potential, including dairy products.

Conclusion

To date this is the first report that describes the presence of native LAB with potential probiotic properties in juçara palm fruits, an important plant for the Atlantic Forest ecosystem. The results of the *in vitro* study indicated that the new LAB isolate, *L. lactis* J7, is a promising probiotic candidate for the development of functional products with distinct advantages over *L. pseudomesenteroides* JF17. *L. lactis* J7 exhibited desirable characteristics demonstrated by its ability to tolerate simulated gastrointestinal conditions, antimicrobial activity against foodborne pathogens and could better adapt to survival and colonization in the intestinal tract according to its aggregation. Furthermore, this study revealed that *L. lactis* J7, by tolerating high concentrations of sodium chloride, could be a potential starter culture in the development of new probiotic food products. However, further studies should be carried out to investigate the adherence properties of these probiotic bacteria using epithelium cell lines and animal models.

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