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ORIGINAL ARTICLE

Leuconostoc mesenteroides SJRP55: a potential probiotic strain isolated from Brazilian water buffalo mozzarella cheese

Aline Teodoro de Paula · Ana Beatriz Jeronymo-Ceneviva · Luana Faria Silva · Svetoslav Dimitrov Todorov · Bernadette Dora G. Mello Franco · Ana Lúcia Barretto Penna

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Abstract The probiotic potential of *Leuconostoc mesenteroides* subsp. *mesenteroides* SJRP55, isolated from water buffalo mozzarella cheese was evaluated. The microorganism presented resistance to stressful conditions that simulated the gastrointestinal tract, and to the best of our knowledge, *Leuconostoc mesenteroides* subsp. *mesenteroides* SJRP55 was the first of this species with the ability to deconjugate bile salts. Tolerance to NaCl was temperature dependent, as well the results obtained by aggregation capacity. The strain presented good adhesion properties, β–galactosidase activity, viability in fermented milk during storage, inactive against *Streptococcus thermophilus* and sensitive to most of the tested antibiotics. Some analgesic medications inhibited the growth of the strain. *Leuconostoc mesenteroides* subsp. *mesenteroides* SJRP55 exhibited in vitro probiotic potential, and it can be better characterized through future in vivo tests. This bacterium presents higher functional properties compared to other studied strains, and therefore, it is a potential candidate for the application as a probiotic strain, which could be used by industries in the manufacture of functional milk-based products.

Keywords Lactic acid bacteria · Therapeutical characteristics · Aggregation · Bile salts · Adhesion properties · Fermented milk

A. T. de Paula · A. B. Jeronymo-Ceneviva · L. F. Silva · A. L. B. Penna (✉)
Food Engineering and Technology Department, UNESP - Sao Paulo State University, Rua Cristovão Colombo, 2265, 15.054-000 São José do Rio Preto, Brazil
e-mail: analucia@ibilce.unesp.br

S. D. Todorov · B. D. G. M. Franco
Faculty of Pharmaceutical Sciences, Department of Food Science and Experimental Nutrition, USP - Sao Paulo University, Av. Prof. Lineu Prestes, 580, bl. 13B, 05508-000 Sao Paulo, Brazil

Introduction

The consumption of functional dairy products that provide health benefits has increased significantly in the last few years, and consequently, the industry has begun looking for strains with probiotic characteristics for future application in functional foods. Probiotics are defined as live, non-pathogenic microorganisms that, when administered in adequate amounts, confer a health benefit to the consumer (FAO/WHO 2002).

The literature describes countless benefits of probiotic cultures. They have been found to enhance the host's immune response, to alleviate symptoms of lactose intolerance, to produce certain vitamins, to be useful in the treatment of many types of diarrhea, to compete with and inhibit pathogenic microorganisms, to reduce cholesterol, the risk of colon cancer, and allergic symptoms, to suppress infection by *Helicobacter pylori*, to improve oral health, and to influence the course of critically ill patients, among other benefits (Adams 2010; Reis et al. 2011; Singh et al. 2011; Amara and Shibl 2013; Azevedo et al. 2013). However, some of the mechanisms by which probiotic strains exert beneficial effects are largely unknown or not well understood (Amara and Shibl 2013).

Some epidemiological studies have shown the beneficial effects of *Leuconostoc* strains. The reduction of acute diarrhea in children was confirmed after the children were fed Indian Dahi, a traditional Indian fermented milk containing *Lactococcus lactis*, *Lactococcus lactis* subsp. *cremoris*, and *Leuconostoc mesentereoides* subsp. *cremoris* (Agarwal and Bhasin 2002). Use of pre- and probiotics have been found to reduce bacterial infection rates after liver transplantation and in patients who have undergone other high-risk surgeries (Rayes et al. 2005, 2009).

To be considered a probiotic culture, the microorganism must be safe (non-pathogenic, absent of virulence genes and antibiotic resistance, and present genetic stability); it must possess technological food qualities (viable during the storage period,

bacteriophage resistance, and ability to be produced on a large scale); it must survive the stress condition(s) of the gastrointestinal tract (GIT); it must be able to adhere and colonize the intestinal cells; and it must present therapeutic benefits. All of these descriptions should be validated and documented, and the replicate results found in the in vitro trials should be confirmed through in vivo assays (Reis et al. 2011; Fontana et al. 2013).

In an attempt to promote health and to ensure the treatment or management of diseases, probiotics are present in many types of foods (Amara and Shibl 2013). The use of lactic acid bacteria (LAB) as probiotic cultures by dairy industries has become more frequent, and it is acceptable to consumers due the production of flavor and aroma by the culture (Shiby and Mishra 2013). Moreover, when present in dairy products, these microorganisms can produce lactic acid and other antimicrobial compounds, such as bacteriocins, which can inhibit undesirable microorganisms, thus extending the shelf-life of the products, and promoting therapeutic, sensory, and technological food benefits (Kos et al. 2007).

Bacteria belonging to the genera *Leuconostoc* are heterofermentative LAB isolated mainly from vegetables, cereal, silage, fruits, wine, fish, meat, and dairy products. However, this microorganism may be involved in the deterioration of some products and, in rare cases, it may also be involved in diseases in immunocompromised patients (Lee et al. 2011).

Some studies on the evaluation of *Leuconostoc mesenteroides* subsp. *mesenteroides* as a potential probiotic culture are still preliminary. Moreover, this microorganism has important technological properties, such as production of dextran, acetaldehyde, diacetyl and acetoin, lipolytic and proteolytic enzymes, low production of acid, and ability to grow under stress conditions (acid, high salt content, and elevated temperature) (Nieto-Arribas et al. 2010). Few research studies have observed the probiotic characteristics of this microorganism (Agarwal and Bhasin 2002; Aswathy et al. 2008; Tamang et al. 2009; Shobharani and Agrawal 2011; Allameh et al. 2012; Seo et al. 2012). These characteristics allow us to understand better the potential probiotic properties of *Leuconostoc mesenteroides* subsp. *mesenteroides*.

The objective of this study was to evaluate the potential probiotic characteristics of *Leuconostoc mesenteroides* subsp. *mesenteroides* SJRP55 isolated from water buffalo mozzarella cheese, including the determination of its viability in co-culture with a commercial starter microorganism in fermented milk during the storage condition.

Materials and methods

Culture media and incubation conditions

Leuconostoc mesenteroides subsp. *mesenteroides* SJRP55, a bacteriocinogenic strain (Paula et al. 2012) was isolated from water buffalo mozzarella cheese and identified by whole 16S rDNA gene sequencing (Silva 2010). The strain was cultured in MRS broth (Difco laboratories, Detroit, MI, USA) at 30 °C and stored at −80 °C with 20 % (v/v) glycerol.

Tolerance to pH, bile and NaCl

A static in vitro model was used to determine the transit tolerance through simulated gastric juice, and the ability of the strain to grow in the presence of bile and NaCl was carried out, according to Todorov et al. (2008). *Leuconostoc mesenteroides* subsp. *mesenteroides* SJRP55 was cultured overnight in MRS broth adjusted to different pH values (3.0, 4.0, 5.0, 6.0, 7.0, 9.0, 11.0, and 13.0), oxbile concentration [0.2, 0.4, 0.6, 1.0, 2.0, and 3.0 % (w/v): Sigma-Aldrich, St Louis, MO, USA] and NaCl concentration [0, 0.5, 1.0, 2.0, 3.0, 5.0, and 10.0 (w/v): Synth, Diadema, São Paulo, Brazil]. All tests were conducted in sterile flat-bottom, 96-well microtiter plates (TPP, Trasadingen, Switzerland). Each well was filled with 180 μl of modified MRS broth (pH, oxbile, and NaCl) and 20 μl of the culture [Optical density at 600 nm ($OD_{600\ nm}$)=0.2]. The strain was incubated at 37 °C for the pH and oxbile tests, and at 5 °C, 30 °C, and 37 °C for the NaCl test. These temperatures were chosen to simulate the optimum growth temperature (30ºC), the natural human body temperature (37ºC), and the refrigerated storage of dairy products (5ºC). Every hour for 12 h, the $OD_{600\ nm}$ was recorded. Experiments were performed in triplicate.

Bile salts deconjugation

Bile salt hydrolase activity was evaluated according to Kumar et al. (2013) with slight modifications. Ten microliters of overnight cultures were spotted onto two different modified MRS agar plates supplemented with 0.5 % (w/v) taurodeoxycholic acid sodium salt (Sigma-Aldrich) or taurocholic acid sodium salt hydrate (Sigma-Aldrich), both with 0.37 g/l of calcium chloride (Synth). The plates were incubated at 30 °C for 72 h. The strains displaying a white precipitation zone surrounding the colonies were considered to be positive.

Auto-aggregation and co-aggregation assays

The cells present in 10 ml of an overnight culture of the *L. mesenteroides* subsp. *mesenteroides* SJRP55 and the tested cultures of *Enterococcus faecalis* ATCC 19443, *L. mesenteroides* subsp. *mesenteroides* UCV10CET (MRS broth, 30 °C), *Listeria innocua* ATCC 33090, and *Listeria monocytogenes* ATCC 7644 (BHI broth [Difco], 30 °C) were washed with sterile saline solution (pH 6.5), harvested by centrifugation (7,000×*g*, 10 min, 20 °C), and diluted to $OD_{660\ nm}$=0.3 (Todorov et al. 2008). Cells were transferred

(1 ml) to a 2-ml sterile Eppendorf tube, and the samples were incubated at 5 °C, 30 °C, and 37 °C. After 1 h, the cell suspension was centrifuged (300×*g*, 2 min, 20 °C) and the $OD_{660\ nm}$ of the supernatant was determined. The percentage of auto-aggregation was calculated using the following formula: $[(OD_0 - OD_{60})/OD_0]\times 100$, where OD_0 refers to initial OD and OD_{60} refers to the OD value measured after 60 min. The co-aggregation trials were performed with overnight cultures of the *L. mesenteroides* subsp. *mesenteroides* SJRP55 in combination with the tested cultures of *Enterococcus faecalis* ATCC 19443, *L. mesenteroides* subsp. *mesenteroides* UCV10CET (MRS broth, 30 °C), and *Listeria innocua* ATCC 33090 and *Listeria monocytogenes* ATCC (BHI broth, 30 °C). The experimental protocol for the study of co-aggregation was the same as that used for auto-aggregation. The co-aggregation trials were performed in the presence of *L. mesenteroides* subsp. *mesenteroides* SJRP55 cells in combination with the cells of the tested strains (500 μl of SJRP55 culture and 500 μl of indicator strain in a sterile plastic cuvette). The percentage of co-aggregation was calculated based on the same formula used for the auto-aggregation analysis. Experiments were conducted in triplicate on two separate occasions.

Cell surface hydrophobicity

The ability of the cell surface to adhere to hydrophobic compounds was evaluated according to the method reported by Doyle and Rosenberg (1995). Cells of the *L. mesenteroides* subsp. *mesenteroides* SJRP55 were harvested (6,700×*g*, 4 °C, 6 min) from overnight culture obtained in MRS at 30 °C, washed twice with phosphate buffer (0.1 mol/l), suspended in the same solution, and the $OD_{580\ nm}$ was measured. Cell suspension (1.5 ml) was added to 1.5 ml of n-hexadecane (Sigma-Aldrich) and vortexed for 2 min. The aqueous and organic phases were allowed to separate for 30 min at room temperature. An aliquot of 1 ml of the aqueous phase was removed to determine the $OD_{580\ nm}$. The percentage of hydrophobicity was calculated based on the same formula used for the auto-aggregation analysis. Experiments were conducted in triplicate.

Adherence to Caco-2 cells

The Caco-2 cell line, known as ATCC HTB-37 (Rio de Janeiro Cell Bank, Rio de Janeiro, Brazil), was routinely cultured (29–31 days) in Dulbecco's modified Eagle's minimum medium (DMEM) (Sigma-Aldrich), supplemented with 20 % (v/v) heat-inactivated fetal bovine serum (Cultilab, Campinas, Brazil), a mixture of penicillin (100 IU/ml) and streptomycin (100 μg/ml) solution (Sigma-Aldrich), and 1 % (v/v) non-essential amino acid solution (Sigma-Aldrich) at 37 °C in an atmosphere of 5 % CO_2 and 95 % air. The adhesion assay was performed as described by Argyri et al. (2013), with modifications. Caco-2 cells were seeded at a concentration of 10^5 cells per well into 24-well tissue culture plates (NEST) and incubated at 37 °C in an atmosphere of 5 % CO_2 and 95 % air (Thermo Fisher Scientific, Waltham, MA, USA) until a confluent monolayer was formed (15–17 days). One day before the performance of the adhesion assays, the medium was replaced, but without antibiotics. Before adhesion, the monolayer was washed once with phosphate-buffered saline (PBS, pH 7.4) to remove all traces of the medium. The *L. mesenteroides* subsp. *mesenteroides* SJRP55 was grown overnight, until it reached the stationary phase in MRS at 30 °C, and it was then washed twice with sterile PBS. Subsequently, approximately 10^8 colony forming units (CFU)/ml was transferred to post-confluent monolayers of Caco-2 cells in the 24-well tissue culture plates and incubated at 37 °C in 5 % CO_2 95 % air atmosphere for 2 h. Cells were then washed at least three times with PBS, in order to remove both the non-adherent bacteria and the cells with adherent bacteria from each well with the addition of 1 ml of Triton X-100 (0.5 %, v/v) (Sigma-Aldrich). The suspension (1 ml) from each well was then transferred to a tube containing 9 ml of sterile saline, serially diluted, and plated on MRS agar in duplicate, in order to determine adhesion ability. Adherence (expressed as a percentage) was calculated using the ratio of the number of bacterial cells that remained attached to the total number of bacterial cells added initially to each well. The experiment was performed in triplicate on two separate occasions.

Enzymatic activity

Enzymatic activities were assayed using an API ZYM kit (BioMérieux, Marcy l'Etoile, France) according to the manufacturer's instructions.

Evaluation of survival of *L. mesenteroides* subsp. *mesenteroides* SJRP55 in fermented milk

In order to study the viability of *L. mesenteroides* subsp. *mesenteroides* SJRP55 and its behavior in co-culture with *Streptococcus thermophilus* TA040 (Danisco, Sassenage, France) in fermented milk, both strains were cultured in reconstituted commercial skim milk (Molico®, Nestlé, Brazil) at 12 % (w/w) of total solids, and the following treatments were used for the tests: (I) *Streptococcus thermophilus* TA040 (St), (II) St+*L. mesenteroides* subsp. *mesenteroides* SJRP55 (St+SJRP55); (III) *L. mesenteroides* subsp. *mesenteroides* SJRP55 (SJRP55). Before the inoculation, the reconstituted milk samples (200 ml) were subject to heat treatment at 90 °C for 10 min in water bath (Marconi, Piracicaba, SP, Brazil), followed by a cooling step done at 10 °C and storage overnight at 4 °C. Experiments were

performed with the initial levels of bacterial populations at 10^8 CFU/ml for both strains. After inoculation, experimental milk samples were incubated at 37 °C in a thermostatically controlled water bath, monitored by Cinac (*Cinétique d'acidification*) system (Ysebaert, Frépillon, France) until the pH 4.6 was reached. After incubation, the fermented milks were cooled on ice bath for 1 h to reach 5 °C, and then they were manually agitated by use of a stainless steel perforated disk-rod that was moved upwards and downwards for 60 s, followed by dispensing the material into 50 ml polypropylene sterile flasks and stored at 4 °C.

The viability of cells was evaluated at 1, 7, 14, 21, and 28 refrigerated storage days. Fermented milks were prepared in two independent assays, resulting in two trials for each type of fermented milk. The cell counts of LAB (*S. thermophilus* TA040 and *L. mesenteroides* subsp. *mesenteroides* SJRP55) were made in duplicate during the refrigerated storage. Homogenized samples (1.0 ml) were subject to serial decimal dilutions in 0.1 % saline solution and plated on M17 agar/37 °C for *S. thermophilus* TA040 and MRS agar supplemented with 1.5 % of oxbile/30 °C (Difco) for *L. mesenteroides* subsp. *mesenteroides* SJRP55, both of them were incubated for 48 h, according to Dave and Shah (1996). Colony forming units (CFU) were enumerated in plates containing 25 to 250 colonies, and cell concentration was expressed as log CFU/ml of fermented milk.

Medications and antibiotics

Resistance of *L. mesenteroides* subsp. *mesenteroides* SJRP55 to drugs from different groups and selected antibiotics was tested according to Todorov et al. (2011). The drugs were purchased in a local drugstore (Sao Jose do Rio Preto, SP, Brazil) and solubilized in sterile water to achieve the desired concentration (Table 1). An 18 h-old culture of SJRP55 was inoculated into 20 ml of MRS soft agar (1.0 %, w/v; Difco) to a final concentration of 10^6 CFU/ml. After solidification, the drugs were diluted separately in 5 ml of sterile distilled water and 10 μl was spotted onto the surface of the agar and incubated at 30 °C for 24 h. Inhibition zones around the spotted drug were checked, and those which presented inhibition zones larger than 2 mm in diameter were tested to determine the minimal inhibitory concentration (MIC). For this test, a serial twofold dilution of the drugs was prepared in sterile water and 10 μl were spotted onto the surface of the 20 mL of MRS soft agar plates that had been previously inoculated with the *L. mesenteroides* subsp. *mesenteroides* SJRP55 (10^6 CFU/ml). The plates were incubated for 24 h at 30 °C and examined for the presence of inhibition zones around the spotted drug. The MIC corresponds to the highest dilution that resulted in inhibition halos of at least 2 mm diameter.

The sensitivity to antibiotics (Table 2) was tested under the same conditions used to test the medications − the spots of drugs were replaced with antibiotics, which were applied using the disc diffusion test (Oxoid, Hampshire, England). The diameters of the inhibition zones surrounding the disks were measured in millimeters.

Results

Tolerance to pH, bile salts, and NaCl

Leuconostoc mesenteroides subsp. *mesenteroides* SJRP55 was more resistant in alkaline pH (9.0-11.0) than under other conditions (3.0–6.0; 13.0). The pH 13.0 resulted in a significant loss of viability. During the incubation period, the pH values 3.0–7.0 promoted a slight interference on the growth of the culture (Fig. 1). *Leuconostoc mesenteroides* subsp. *mesenteroides* SJRP55 grew well in all concentrations of bile salts tested (between 0.2 % and 3.0 %, Fig. 2). A white precipitation zone surrounding the colony showed the ability of *L. mesenteroides* subsp. *mesenteroides* SJRP55 culture to deconjugate sodium taurodeoxycholic acid sodium salt and taurocholic acid.

The presence of different concentrations of NaCl and the incubation temperature were limiting factors for the growth of *L. mesenteroides* subsp. *mesenteroides* SJRP55, which has survived in all temperatures analyzed (5ºC, 30ºC, 37ºC), but the growth was reduced, mainly at 5ºC (Fig. 3). Moreover, an increase in NaCl concentration resulted in a reduction in the growth and survival of the culture which has tolerated up to 5 % NaCl at 30ºC and 37ºC.

Auto-aggregation and co-aggregation assays

The highest auto-aggregation rates of *L. mesenteroides* subsp. *mesenteroides* SJRP55 (85.64 %) was observed at 30ºC (Fig. 4). When the tests were performed at 37ºC and 5ºC, similar auto-aggregation values were obtained (51.05 % and 51.67 %), respectively. In addition, *L. mesenteroides* subsp. *mesenteroides* SJRP55 presented a greater ability to auto-aggregate than the indicator cultures at 30ºC. Among the indicator cultures, the most auto-aggregative strain was *Listeria innocua* at 5ºC (60.71 %) and at 30ºC (80.52 %), and *Enteroccoccus faecalis* at 30ºC (52.60 %). *Leuconostoc mesenteroides* subsp. *mesenteroides* SJRP55 was found to possess the ability to co-aggregate with the indicator strains tested. The highest value of co-aggregation was with *Enteroccoccus faecalis* (90.37 %) at 30ºC and the lowest

Table 1 Antibiotic susceptibility of *Leuconostoc mesenteroides* subsp. *mesenteroides* SJRP55

Antibiotic (μg per disc)	Classification[b]	Inhibition zone (mm)
Amikacin 30	Aminoglycoside (inhibits protein synthesis)	19
Gentamicin 10		20
Kanamycin 30		16
Tobramycin 10		17
Amoxicillin/clavulanic acid 30	β-Lactam (interfere with bacteria cell wall synthesis)/β-Lactam (inhibits the β-lactamase)	23
Cefuroxime 30		28
Oxacillin 5		16
Ampicillin 10	Penicillin/β-Lactam (interfere in the bacteria cell wall synthesis)	24
Penicillin G 5		20
Bacitracin 10	Cyclic polypeptide (inhibits bacteria cell wall synthesis)	27
Cefaclor 30	Second-generation cephalosporin/β-Lactam (interferes with bacterial cell wall synthesis)	16
Cefotaxim 30		22
Cefepime 30	Fourth-generation cephalosporin/β-Lactam (interferes with bacterial cell wall synthesis)	16
Ceftazidim 30	Third-generation cephalosporin/β-Lactam (interferes with bacterial cell wall synthesis)	11
Ceftiofur 30		22
Ceftriaxone 30		17
Ciprofloxacin 5	Fluoroquinolone (inhibits bacterial topoisomerase II)	13
Clarithromycin 15	Macrolide (inhibits protein synthesis)	25
Erythromycin E 10		26
Chloramphenicol 30	Chloramphenicol (prevents peptide bond formation – inhibits protein synthesis)	26
Doxycycline hydrochloridric 30	Tetracycline (inhibits protein synthesis)	31
Minocycline 30		32
Tetracycline 30		28
Linezolid 30		31
Florfenicol 30	Synthetic compound similar to chloramphenicol (prevent the linkage of peptides – inhibits protein synthesis	28
Imipenem 10	Carbapenem/β-Lactam (interferes with bacterial cell wall synthesis)	21
Moxifloxacin 5	Fourth-generation synthetic fluoroquinolone (inhibiting DNA gyrase)	23
Nalidixic acid 30[a]	*Synthetic quinolone antibiotic* (*inhibiting DNA gyrase*)	0
Nitrofuranton 10	Nitrofuran derivative (nucleic acid inhibitor)	10
Ofloxacin 5	Lincosamide (inhibits protein synthesis)	19
Rifampicin 5	Semi-synthetic compound derivate from *Amycolatopsis rifamycinica*	24
Sulfamethoxazole/trimethoprim 25	*Sulfonamide* (*inhibits folate synthesis*)	0
Sulfonamide 300	*Inhibits folate synthesis*	0
Teicoplanin 30	*Glicopeptides/β-Lactam* (*interferes with bacterial cell wall synthesis*)	0
Vancomycin 5		0

[a] Italic: antibiotics that did not affect the growth of *L. mesenteroides* subsp. *mesenteroides* SJRP55

[b] Todorov et al. (2012)

was with *L. mesenteroides* subsp. *mesenteroides* UCV10CET (42.42 %) at 5ºC.

Cell surface hydrophobicity

Leuconostoc mesenteroides subsp. *mesenteroides* SJRP55 presented 59.12 % cell surface hydrophobicity, measured using the interaction with *n*-hexadecane to simulate the ability to adhere to the intestinal epithelium.

Adherence to Caco-2 cells

Leuconostoc mesenteroides subsp. *mesenteroides* SJRP55 presented high adhesion (91.5 %) to Caco-2 cells.

Enzymatic activities

Leuconostoc mesenteroides subsp. *mesenteroides* SJRP55 exhibited β–galactosidase activity. From the API ZYM kit test, it

Table 2 Effect of medications on the growth of *Leuconostoc mesenteroides* subsp. *mesenteroides* SJRP55

Brazilian commercial name	Concentration (mg/ml)	Active substance	Medication class	Inhibition zone (mm) [MIC (mg/ml)]
Advil	40	Ibuprofen	Analgesic	23 [40.0]
Buscopan	2	Butylscopolamine	Analgesic, antispasmodic	27 [12.5]
	50	Metamizole		
Cloridrato de prometazina	5	Promethazine hydrochloride	Antihistaminic	14 [5.0]
Cozaar	20	Losartan	Antihypertensive	13 [20.0]
Dipirona sódica	100	Metamizole	Analgesic	22 [3.0]
Dorflex	60	Metamizole	Analgesic, anti-inflammatory, and muscle relaxant	21 [1.8]
	10	Caffeine		
	7	Orphenadrine citrate		
Ibuprofene Biogaran	40	Ibuprofen	Anti-inflammatory	23 [40.0]
Lisador	100	Metamizole	Analgesic, antispasmodic intestinal, uterine antispasmodic, and antipyretic	19 [3.0]
	2	Adifenine hydrochloride		
	1	Promethazine hydrochloride		
Metotrexato	0.5	Methotrexate Sodium	Antimetabolic	35 [0.5]
Migraliv	0.2	Dihydroergotamine mesylate	Anti-migraine	29 [87.5]
	70	Metamizole		
	20	Caffeine		
Neosaldina	60	Metamizole	Analgesic and antipyretic	27 [30.0]
	6	Isometheptene mucate		
	6	Anhydrous caffeine		
Novalgina	100	Metamizole	Analgesic and antipyretic	25 [6.25]
Metotrexato	0.5	Methotrexate Sodium	Antimetabolic	35 [0.5]

The following commercial drugs have no effect on the growth of *L. mesenteroides* subsp. *mesenteroides* SJRP55: AAS adulto (acetylsalicylic acid, analgesic, and antipyretic, at 100.0 mg/ml); Aldactone (spironolactone, diuretic, at 5.0 mg/ml); Ansiopax (*Piper methysticum*, anxiolytic, at 46.8 mg/ml); Biprofenid (ketoprofen, anti-inflammatory, analgesic, at 30.0 mg/ml); Cardilol (carvedilol, antianginal, and antihypertensive, at 1.25 mg/ml); Celebra (celecoxib, anti-inflammatory, at 40.0 mg/ml); Cezarette (desogestrel, contraceptive, at 15.0 mcg/ml), Clonotril (clonazepam, anxiolytic, at 0.1 mg/ml); Cloridrato de fexofenadine (fexofenadine hydrochloride, anti-allergy, at 36.0 mg/ml); Cloridrato de propranolol (propranolol hydrochloride, antihypertensive, at 8.0 mg/ml); Cloxazolam (anxiolytic and sedative, at 0.4 mg/ml); Decongex plus (phenylephrine hydrochloride and brompheniramine maleate, decongestant of the upper respiratory tract, at 3.0 and 2.4 mg/ml); Diasec (loperamide hydrochloride, anti-diarrhoeal, at 0.4 mg/ml); Diovan amlo fix (valsartan, amlodipine, antihypertensive, at 32.0 and 1.0 mg/ml); Doliprane (paracetamol, analgesic, antipyretic, at 200.0 mg/ml); Dramin B6 (dimenhydrinate and pyridoxine hydrochloride, antiemetic, at 10.0 and 2.0 mg/ml); Ebastel (ebastine, antihistamynic, at 2.0 mg/ml); Enalprin (maleat enalapril, antihypertensive, at 4.0 mg/ml); Flamador (ketoprofen, analgesic, anti-inflammatory, antirheumatic, at 10.0 mg/ml); Levoid (levothyroxine sodium, treatment of thyroid problems, at 17.6 mcg/ml); LipLess (ciprofibrate, anti-hypertriglyceridaemia, at 20.0 mg/ml); Loratadina (loratadine, antihistamine, antiallergic, at 2.0 mg/ml); Maleato de enalapril (maleat enalapril, antihypertensive, at 2.0 mg/ml); Maracugina (*Passiflora alata*, *Erythrina mulungu*, *Crataegus oxyacantha*, neuro-sedative, at 5.0 and 2.5 mg/ml); Maxsulid (nimesulide beta-cyclodextrin, analgesic, anti-inflammatory, and antipyretic, at 80.0 mg/ml); Meloxicam (meloxicam, anti-inflammatory, at 3.0 mg/ml); Meticorten (prednisone, anti-inflammatory, at 4.0 mg/ml); Metiocolin B12 (DL-methionine and inositol, hepatoprotective, choline chloride and cobalamin, at 20.0, 10.0, 5.0 m mg/ml and 0.4 mcg/ml); Mioflex (paracetamol, carisoprodol, fenilbutazone, analgesic, anti-inflammatory, muscle relaxant, at 60.0, 30.0 and 15.0 mg/ml); Miosan (cyclobenzaprine hydrochloride, muscle relaxant, at 1.0 mg/ml); Motilium (domperidone, antiemetic, at 2.0 mg/ml); Omepramedi (omeprazole, proton pump inhibitor, at 4.0 mg/ml); Paracetamol (paracetamol, analgesic, antipyretic, at 150.0 mg/ml); Plasil (metoclopramide hydrochloride, antiemetic, at 2.0 mg/ml); Plaq (clopidogrel bisulfate, antihypertensive, at 15.0 mg/ml); Prelone (prednisolone, corticosteroid, at 1.0 mg/ml); Profenid enterico (ketoprofen, anti-inflammatory, analgesic, antipyretic, at 20.0 mg/ml); Primosiston (ethinyl estradiol and norethisterone acetate, antihemorrhagic, at 0.4 and 0.002 mg/ml); Resfenol (paracetamol, chlorpheniramine maleate and phenylephrine hydrochloride, analgesyc and antipyretic, at 80.0 and 0.8 mg/ml); Rupafin (rupatadine fumarate, antiallergic, at 2.56 mg/ml); Selozok (metoprolol succinate, antihypertensive, at 10.0 mg/ml); Sinvalip (simvastatin, hypolipidemic, at 4.0 mg/ml); Somalgin cardio (acetylsalicylic acid, analgesic and antipyretic, at 20.0 mg/ml); Spasfon LYOC (phloroglucinol, antispasmodic, at 16.0 mg/ml); Tamisa 30 (gestodene and ethinyl estradiol, contraceptive, at 15.0 and 0.006 mcg/ml); Toragesic (kerotolac trometamol, analgesic, at 2.0 mg/ml); Transamin (tranexamic acid, antihemorrhagic, at 50.0 mg/ml); Tylenol (paracetamol and pseudoephedrine chloridrate, analgesic and antipyretic, at 100.0 and 6.0 mg/ml); Tylex (paracetamol and codeine fosfate, analgesic and antipyretic, at 100.0 and 6.0 mg/ml); Vasativ (cilostazol, antiplatelet, at 20.0 mg/ml); Vertix (flunarizine dihydrochloride, calcium channel blocker, at 2.0 mg/ml)

was also observed that *L. mesenteroides* subsp. *mesenteroides* SJRP55 presented a weak enzymatic activity for leucine arylamidase, cystine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase. It was not detected at

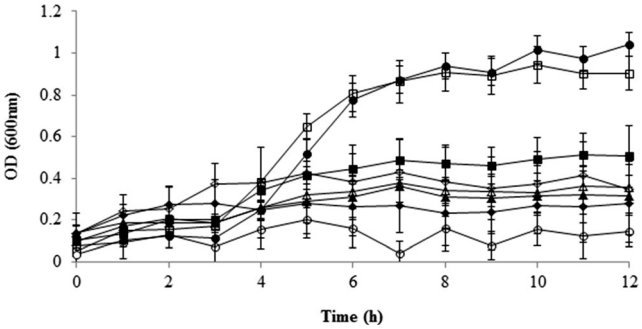

Fig. 1 Growth of *Leuconostoc mesenteroides* subsp. *mesenteroides* SJRP55 in MRS broth at pH levels adjusted from 3.0−13.0, shown as OD (600 nm) measurements. The results are represented as an average of three readings. (♦) 3.0, (◊) 4.0, (▲) 5.0, (Δ) 6.0, (■) 7.0, (□) 9.0, (●) 11.0, (○) 13.0

studied conditions in the production of proteinase, esterase, lipase and β-glucuronidase enzymes (data not shown).

Evaluation of co-survival of *L. mesenteroides* subsp. *mesenteroides* SJRP55 and *Streptococcus thermophilus* TA040 in fermented milk

The viability of the tested LAB strains (*L. mesenteroides* subsp. *mesenteroides* SJRP55 and *S. thermophilus* TA040) was stable during the storage condition (Fig. 5). In the beginning of the tested period (first day of analysis) *L. mesenteroides* subsp. *mesenteroides* SJRP55 showed a low decrease in the cell counts; however, it recovered its growth during the refrigerated storage. When both strains (*L. mesenteroides* subsp. *mesenteroides* SJRP55 and *S. thermophilus* TA040) were co-cultured, no inhibitory effect between them was recorded.

Medications and antibiotics

In order to address certain prerequirements of potential probiotic strain, the safety of the probiotic strain was evaluated among the 35 tested antibiotics. *Leuconostoc mesenteroides* subsp. *mesenteroides* SJRP55 was found to be resistant to only five antibiotics: nalidixic acid, sulfamethoxazole/trimethoprim, sulfonamide, teicoplanin, and vancomycin (Table 1).

Among the 65 tested drugs, 12 affected the growth of *L. mesenteroides* subsp. *mesenteroides* SJRP55, and most of them were analgesic and anti-inflammatory medications containing ibuprofen, butylscopolamine, metamizole, caffeine, orphenadrine citrate, and isometheptene mucate (Table 2). In addition, Cozaar (Losartan) was the only antihypertensive drug that affected the survival of the probiotic bacteria. The MICs found for the analgesic drugs. Dipirona sódica (3.0 mg/ml), Dorflex (1.8 mg/ml), Lisador (3.0 mg/ml), and Novalgina (6.25 mg/ml) were very low, comparing to the concentration commonly used by these drugs.

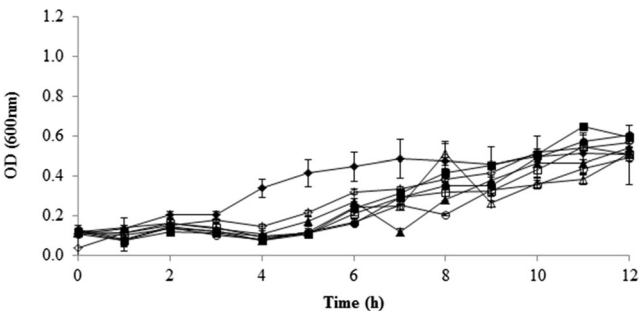

Fig. 2 Growth of *Leuconostoc mesenteroides* subsp. *mesenteroides* SJRP55 in MRS broth supplemented with 0–3.0 % bile salts, shown as OD (600 nm) measurements. The results are represented as an average of three readings. (♦) 0, (◊) 0.2, (▲) 0.4, (Δ) 0.6, (■) 0.8, (□) 1.0, (●) 2.0, (○) 3.0 %

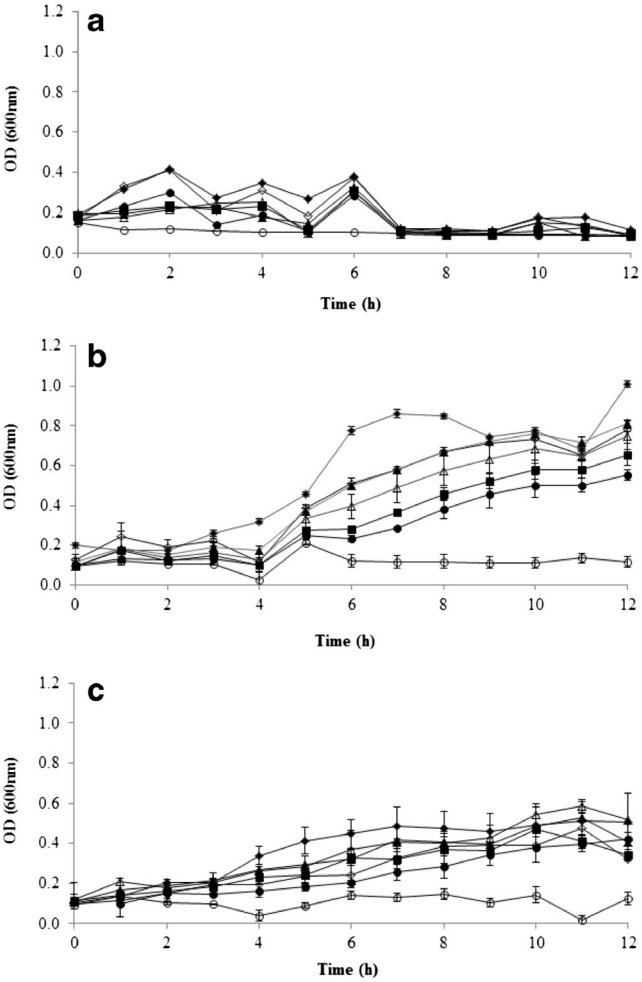

Fig. 3 Growth of *Leuconostoc mesenteroides* subsp. *mesenteroides* SJRP55 in MRS broth with 0–10·0 % of NaCl at 5ºC **a**, 30ºC **b** and 37ºC **c**, shown as OD (660 nm) measurements. The results are represented as an average of three readings. (♦) 0, (◊) 0.5, (▲) 1.0, (Δ) 2.0, (■) 3.0, (●) 5.0, (○) 10.0 %

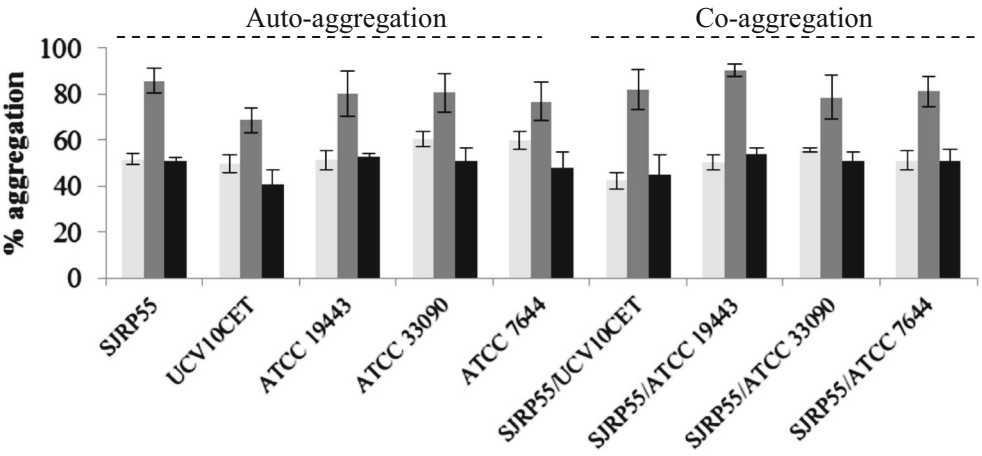

Fig. 4 Auto-aggregation and co-aggregation of *Leuconostoc mesenteroides* subsp. *mesenteroides* SJRP55, *L. mesenteroides* subsp. *mesenteroides* UCV10CET, *Enterococcus faecalis* ATCC 19443, *Listeria innocua* ATCC 33090, and *Listeria monocytogenes* ATCC 7644 at 5ºC (light gray bar), at 30ºC (dark gray bar), and at 37ºC (black bar) expressed as percentage. Each result is represented as an average of three readings

Discussion

The in vitro study showed that *Leuconostoc mesenteroides* subsp. *mesenteroides* SJRP55 can survive against the stress conditions found in the gastrointestinal tract. To be considered a probiotic culture, some criteria should be fulfilled, including the ability to tolerate and survive the acidic environment of the stomach and the bile salts present in the small intestine. *Leuconostoc mesenteroides* subsp. *mesenteroides* isolated from fish intestine survived better under neutral conditions, which is similar to the neutral condition present in the intestines (Allameh et al. 2012). Previous studies also reported that LAB were able to grow and survive at low pH levels (Mishra and Prasad 2005; Divya et al. 2012). However, the tolerance of gastric transit has been found to be variable among the strains (Vinderola and Reinheimer 2003). It is important to point out that the in vitro trials involving pH, bile salts, and NaCl tolerance cannot predict patterns of behavior in the human body. This is because most methodologies used to analyze the potential probiotic strains in stressful conditions are static models, which cannot foresee the gradual changes of pH values and bile salts in the GIT (Todorov et al. 2011).

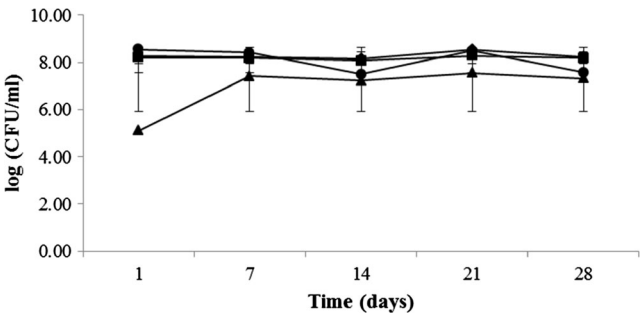

Fig. 5 Cell counts of *Streptococcus thermophilus* (St), *S. thermophilus*+ *L. mesenteroides* subsp. *mesenteroides* SJRP55 (St+SJRP55) and *L. mesenteroides* subsp. *mesenteroides* SJRP55 (SJRP55) in fermented milk. The results were represented as two independent assays resulting in two trials for each type of milk. (♦) *S. thermophilus* (St) in the milk fermented by *S. thermophilus*, (■) *S. thermophilus* (St) in the milk fermented by *S. thermophilus* + *L. mesenteroides* SJRP55, (▲) *L. mesenteroides* subsp. *mesenteroides* SJRP55 (SJRP55) in the milk fermented by *S. thermophilus* + *L. mesenteroides* SJRP55, (●) *L. mesenteroides* subsp *mesenteroides* SJRP55 in the milk fermented by *L. mesenteroides* subsp *mesenteroides*

The physiological concentration of human bile varies and depends on race, physiological conditions, and gender. Studies have shown that all probiotic strains should be able to grow and survive in the presence of up to 0.3 % bile salts (Divya et al. 2012). The ability to survive at different bile salt concentrations was confirmed by *L. mesenteroides* subsp. *mesenteroides* SJRP55. According to Ouwehand et al. (1999), Aswathy et al. (2008), and Meira et al. (2012), LAB isolated from different sources were also relatively tolerant to bile salts. In addition, Allameh et al. (2012) and Todorov et al. (2008) reported that bile salt affected the growth rate of *L. mesenteroides* subsp. *mesenteroides* and limited its viability. The ability to survive at different bile salts concentrations was confirmed by *L. mesenteroides* subsp. *mesenteroides* SJRP55. This positive behavior can increase the possibility of this microorganism to colonize and grow in gut condition, which can activate its therapeutical characteristics.

Many attempts have been made to demonstrate the reduction of cholesterol concentrations in human blood using bile salt hydrolases from LAB strains, but it has not yet been proved (Kumar et al. 2013). Deconjugation of bile salts by LAB increases the demand for cholesterol which, in turn, prompts the synthesis of more bile salts in the liver. This process may lead to a reduction in serum cholesterol (Kumar et al. 2013). Despite different species of LAB can present the ability to deconjugate bile salts (Vinderola and Reinheimer 2003), there are currently no published studies on bile salt deconjugation by *Leuconostoc mesenteroides* strains. Therefore, to the best of our knowledge *Leuconostoc mesenteroides* subsp. *mesenteroides* SJRP55 is the first of this species that presents this property, emphasizing its functional qualities as a potential probiotic strain.

Most LAB are halotolerant, which is an important characteristic for their use in dairy products, especially cheeses, and *Leuconostoc* can grow in the presence of 7.0 % NaCl (Hemme

and Foucaud-Scheunemann 2004). *Leuconostoc mesenteroides* subsp. *mesenteroides* normally has a very limited growth at 5ºC and in the presence of NaCl and the best temperature condition observed in our study was at 30ºC, probably because this is the optimum growth temperature for this microorganism.

The aggregation phenotype can help probiotic cultures to adhere, colonize the GIT, and modulate the immune system (Ouwehand and Vesterlund 2004). The strain evaluated in the present study was found to have higher auto-aggregation values than the indicator microorganisms. This result suggests the specific affinity of *L. mesenteroides* subsp. *mesenteroides* SJRP55 to the GIT, probably due the formation of biofilms, which can help the colonization of SJRP55 strain to the epithelial intestine cells. Similar results were reported by Collado et al. (2007) and Xu et al. (2009) with different species of potential probiotic LAB. Co-aggregation has been related to the ability of potential probiotic strain to interact closely with pathogens (Collado et al. 2007). This characteristic can increase the competition of receptor epithelial intestine cells and may decrease the presence of undesired microorganism in the intestine due the production of antimicrobial compounds or other factors. The results of co-aggregation observed here were strain-specific and incubation temperature-dependent. A downside is that high co-aggregation values may be a potential virulence factor, through which genetic material can be transferred by conjugation (Hemme and Foucaud-Scheunemann 2004).

Hydrophobicity is one of the physicochemical properties that can facilitate the first contact between the microorganisms and the host cells (Shobharani and Agrawal 2011). These results should be interpreted with caution because the adherence feature to intestines does not necessarily mean an in vivo adhesion would occur (Bautista-Gallego et al. 2013). Moreover, cell surface hydrophobicity is strain-specific and the presence of different nutrients or carrier food matrices may influence the expression of adhesion genes in the microorganisms (Ouwehand and Vesterlund 2004; Raghavendra and Halami 2009). The results obtained in the hydrophobicity tests for *L. mesenteroides* subsp. *mesenteroides* SJRP55 were higher than some other strains reported in the literature (Aswathy et al. 2008; Raghavendra and Halami 2009): LAB isolated from fermented vegetables, sourdough, milk products, and sheep and human excreta (23.0 to 73.0 %) and *Pediococcus pentosaceus* CFR R38 and CFR R35, and *Lactobacillus rhamnosus* GG ATCC 53510 isolated from different sources (44.8 to 59.0 %). Results also differed in the case of *Leuconostoc paramesenteroides* isolated from cheddar cheese (46.11 %) (Shobharani and Agrawal 2011). Moreover, different compounds commonly used to evaluate the hydrophobicity (*n*-hexadecane, xylene and toluene) can lead to different results. In addition, the hydrophobicity property can be related to the auto-aggregation and co-aggregation abilities of the strains.

The adhesion mechanisms are not fully understood (Argyri et al. 2013), and the ability to adhere to the Caco-2 cells seems to be strain-dependent. Few studies discuss the adhesion properties of *Leuconostoc mesenteroides* species; however, the bacterial adhesion values increase with production of exopolysaccharides (EPS). (Ryu and Chang 2013). Moreover, the production of this material is frequent in *Leuconostoc mesenteroides* species (Giraffa 2012; Bendimerad et al. 2012). *Leuconostoc* spp. is not a common colonizer genus in the intestinal tract (Hemme and Foucaud-Scheunemann 2004). However, studies suggest that the intake of live and also heat-killed *Leuconostoc mesenteroides* 1RM3 might prevent *Listeria monocytogenes* entero-gastric administrated from invading Caco-2 cells and infecting A/J mice (Nakamura et al. 2012). This adherence characteristic is also present in other isolated LAB (Perea Velez et al. 2007; Deepika et al. 2011; Argyri et al. 2013). The ability to adhere to mucosal surfaces in the intestine plays an important role in defining a probiotic culture. The colonization of the intestine by probiotic strains can generate beneficial biological responses: they can influence immune system and increase the competition with pathogens in the intestine (Adams 2010). The in vitro adhesive proprieties of *L. mesenteroides* subsp. *mesenteroides* SJRP55 can indicate that the strain is able to adhere to the intestine of the host and may be able to activate the genes that encode antimicrobial compounds, such as bacteriocins, which can act against pathogens present in the GIT. Moreover, previous studies showed that *L. mesenteroides* subsp. *mesenteroides* SJRP55 possesses anti-*Listeria* activity (Paula et al. 2012).

Some consumers of dairy products are lactose-intolerant, a condition which causes discomfort after the digestion of milk (Raghavendra and Halami 2009). The *β*–galactosidase enzyme hydrolyses lactose to galactose and glucose, which aids lactose digestion in the intestine. When people have limited digestion or are lactose intolerant, the consumption of dairy products causes discomfort, gas retention, and flatulence. Since LAB cultures can produce this enzyme, it is becoming important for the dairy industry to explore this property in order to help lactose-intolerant consumers. Previous studies with *Lactobacillus delbrueckii* subsp. *lactis* (Guglielmotti et al. 2007), *Pediococcus pentosaceus* and *Lactobacillus rhamnosus* (Raghavendra and Halami 2009), *Leuconostoc paramesenteroides* (Shobharani and Agrawal 2011) and *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus casei*, and *Lactobacillus parabuchneri* (Meira et al. 2012) also revealed *β*–galactosidase activity; however, this characteristic seems to be uncommon in *Leuconostoc mesenteroides* species.

The knowledge of the enzymatic profile of the future probiotic strains is an important point, since some enzymes, such as β-glucuronidase may be related to toxic reactions and formation of harmful metabolites. *Leuconostoc mesenteroides* subsp. *mesenteroides* SJRP55 was negative for β-glucuronidase activity based on the results obtained from API ZYM kit. However, various studies have suggested that the enzymatic activity of *Leuconostoc mesenteroides* cultures is strain-specific (Thapa et al. 2006; Ryu and Chang 2013).

Probiotic cultures have been exploited extensively by dairy industry as a tool for the development of novel functional products (Vasiljevic and Shah 2008). The viability of probiotic cultures is a major concern, since it can affect the probiotic characteristics of the products. In contrast, studies have shown that probiotic dead cells also exhibit beneficial effects to the host (Adams 2010). In our study, tests used to evaluate the stability of *L. mesenteroides* subsp. *mesenteroides* SJRP55 in fermented milk during the storage condition, alone and in co-culture with *S. thermophilus* TA040, showed that this microorganism can survive at desirable levels during fermentation and storage period, and it is a good candidate as a probiotic strain for the production of fermented milk products. Moreover, the use of *L. mesenteroides* subsp. *mesenteroides* SJRP55 in co-culture with *S. thermophilus* TA040 showed that this microorganism can be applied as an adjunct culture in fermented milk.

The production of exopolyssacharides, a common compound produced by *Leuconostoc* species can improve the viscosity, texture, and mouthfeel of dairy products (Hemme and Foucaud-Scheunemann 2004; Ruas-Madiedo et al. 2005). We need to underline that in the industrial production of fermented milk products, including yoghurt, viscosity and texture are critical characteristics for consumers' acceptance of the new products on the market.

Some authors have been studying the technological characteristics of *Leuconostoc mesenteroides* (Nieto-Arribas et al. 2010; Cardamone et al. 2011;), and the presence and behavior of this microorganism in the microbial ecology of kefir grains (Hsieh et al. 2012); however up to date, the application of *Leuconostoc mesenteroides* as both as a potential probiotic and bacteriocinogenic strain has not been reported yet.

Several studies have demonstrated the probiotic effect of different strains of *Leuconostoc mesenteroides*. However, even though this microorganism is often isolated in cheeses, few studies highlight the probiotic potential and the microbial behavior of this microorganism when present in these products. In this study, the probiotic effects showed by *L. mesenteroides* subsp. *mesenteroides* SJRP55 indicate that this microorganism can improve the therapeutical product's claim.

The few antibiotics to which the *L. mesenteroides* subsp. *mesenteroides* SJRP55 showed resistance are used for human infections and, depending on the classification, can act against Gram-positive or Gram-negative pathogenic bacteria. Antibiotic resistance is a worldwide public health problem (Ammor and Mayo 2007). The concern over this problem is in part due to the excessive and indiscriminate use of these compounds in humans, agriculture, and livestock. Moreover, LAB, pathogenic bacteria, and opportunistic bacteria can acquire and/or transmit antibiotic resistance and virulence genes by transposons and plasmids, resulting in conjugation or transformation (Grattepanche et al. 2008). Some microorganisms can be intrinsically resistant to antibiotics. In general, *Leuconostoc* sp. has an intrinsic resistance to vancomycin due to particular characteristics of its cell wall, which presents D-lactate instead of a D-alanine in the peptidoglycan (Hemme and Foucaud-Scheunemann 2004). However, no cases of infection by consumption of dairy products containing *Leuconostoc* spp. have been reported, which demonstrate its safety. Moreover, LAB resistant to antibiotics can proliferate in the gut and maintain microbial balance, thereby reducing the levels of opportunistic microorganisms. Previous studies showed that *Leuconostoc* spp. is resistant to fosfomycin, "old" quinolones, and glycopeptides; it is also susceptible to or leads to intermediate sensitivity to macrolides and tetracyclines (Hemme and Foucaud-Scheunemann 2004). *Leuconostoc mesenteroides* sp. *mesenteroides* was resistant to streptomycin and intermediate to amoxicillin and kanamycin (Allameh et al. 2012), whereas *Leuconostoc paramesenteroides* isolated from kimchi was resistant to tetracycline, gentamicin, neomycin, streptomycin and sulfisoxazole (Shobharani and Agrawal 2011). In summary, antibiotic resistance seems to be strain-dependent and related to the environment in which the strain was isolated. A subject of concern regarding LAB as potential probiotic strains is the transference of resistance genes to pathogenic bacteria present in the GIT, which may pose a risk for the host, particularly in the case of immunocompromised people, the elderly, pregnant women, and newborns (Aymerich et al. 2006; Devirgiliis et al. 2011).

Nowadays, lifestyle, stress, and inadequate food intake are raising the consumption of different groups of medications for pain and other kinds of illness. However, many consumers undergoing these therapies are not aware of the side effects of these compounds. In our study, the in vitro tests showed that different medications can inhibit *L. mesenteroides* subsp. *mesenteroides* SJRP55. Most of the tested analgesic, antipyretic, and anti-inflammatory medications are commonly used by people of different ages, from babies to the elderly. Besides this, most of them are freely commercialized, without medical prescription. The negative effect of these drugs against potential probiotic LAB seems to be common, and has been observed in other studies (Todorov et al. 2011, 2012). Moreover, the antihypertensive medication analyzed in our study, Cozaar, is used in long-term treatments for a certain chronic disease. When compared to other antihypertensives, the substance present in this drug seems to be responsible for the inhibition of the *L. mesenteroides* subsp. *mesenteroides* SJRP55.

The minimal inhibitory concentration (MIC) values (Table 2) play an important role in the proper evaluation of the effect of medications on probiotic bacteria (Todorov et al. 2012). The presence of the compound metamizole in analgesic and anti-inflammatory drugs may be the responsible for the low MIC values found in the in vitro test. Metamizole or dypirone is a nonsteroidal anti-inflammatory agent that is prohibited in most industrialized countries because of the risk of fatal agranulocytosis, but it is widely used in Latin

America, Africa, and Asia. This compound binds to neutrophil membranes, creating a novel antigen that induces antibody formation. The resultant immune response causes both peripheral and bone marrow cell lysis (Bonkowsky et al. 2002; Hedenmalm and Spigset 2002; Yiğit and Soyuncu 2012). These results are cause for concern, because the intake of these drugs in Brazil is very frequent at all ages for different therapies. The medications known commercially in Brazil as Dorflex and Neosaldina did not affect the growth of *Lactobacillus casei* Shirota or *Lactobacillus casei* LC01 (Carvalho et al. 2009).

Drugs taken orally are absorbed in the small intestine and the amount of medication that reaches the bloodstream depends on how much is absorbed through the gastrointestinal tract. Enzymes present in the gastrointestinal tract metabolize the drugs, as well as those released by the microbiota. Drugs also interact with foods and beverages, reducing or increasing the amount that gets absorbed, depending on the case. Considering this drug metabolism, more in vivo studies should be done to understand better the interaction between medication and probiotic potential cultures.

Finally, this is the first study with *L. mesenteroides* subsp. *mesenteroides* SJRP55 isolated from water buffalo mozzarella cheese that showed desirable probiotic characteristics using in vitro testing (resistance to stressful conditions that simulated the GIT, ability to deconjugate bile salts, high tolerance to NaCl and aggregation capacity, good adhesion properties, β–galactosidase activity, and low resistance to antibiotics) and viability in fermented milk under storage conditions. *Leuconostoc mesenteroides* subsp. *mesenteroides* SJRP55 presents higher functional properties compared to other studied strains, and therefore, it is a potential candidate for further in vivo investigation studies. These studies will allow us to understand better the strain's potential health benefits, as well as its possible industrial applications for the development of functional foods.

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Conflict of interest The authors declare that there is no conflict of interest.

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