Biotechnol Lett
https://doi.org/10.1007/s10529-018-2572-x

ORIGINAL RESEARCH PAPER

Lactobacillus spp. impair the ability of *Listeria monocytogenes* FBUNT to adhere to and invade Caco-2 cells

P. Castellano · M. Pérez Ibarreche · L. Longo Borges · F. C. Niño Arias · G. R. Ross · E. C. Pereira De Martinis

Received: 25 January 2018 / Accepted: 18 May 2018

Abstract
Objectives The objective of this study was to evaluate the ability of *Lactobacillus curvatus* CRL705, CRL1532, and CRL1533 and *Lactobacillus sakei* CRL1613 to survive under simulated gastrointestinal conditions. Moreover, a microencapsulation approach was proposed to improve gastrointestinal survival. Finally, experiments were performed to demonstrate that *Lactobacill*us spp. can modulate the ability of *Listeria monocytogenes* FBUNT to adhere to and invade Caco-2 cells.
Results *Lactobacillus* strains were encapsulated in alginate beads to enhance the survival of bacteria under in vitro gastrointestinal conditions. All strains hydrolyzed bile salts using chenodeoxycholic acid as a substrate and adhered to Caco-2 cells. Cell-free supernatants (CFSs) showed antimicrobial activity against *L. monocytogenes* as demonstrated by agar diffusion assays. The average percentages of *L. monocytogenes* adhesion decreased from 67.74 to 41.75 and 38.7% in the presence of 50 and 90% (v/v), respectively, for all CFSs tested. The highest concentrations of CFSs completely inhibited the *L. monocytogenes* invasion of Caco-2 cells.
Conclusions The studied *Lactobacillus* strains have protective effects against the adhesion and invasion of *L. monocytogenes* FBUNT. Alginate encapsulation of these bacteria improved gastrointestinal tolerance such that they could be further studied as potential probiotics against intestinal pathogenic bacteria.

Keywords Adhesion · Invasion · *L. monocytogenes* · Probiotic

P. Castellano (✉) · M. Pérez Ibarreche
Centro de Referencia para Lactobacilos (CERELA-CONICET), Chacabuco 145, 4000 Tucumán, Argentina
e-mail: patricia@cerela.org.ar

L. Longo Borges · F. C. Niño Arias · E. C. P. De Martinis
Faculdade de Ciências Farmacêuticas de Ribeirão Preto – USP, Av. do Café s/n, Monte Alegre, Ribeirão Preto, SP 14040-903, Brazil

G. R. Ross
Instituto de Biotecnología farmacéutica y alimentaria (INBIOFAL-CONICET), Av Kichner, 4000 Tucumán, Argentina

Introduction

Listeria monocytogenes is primarily a foodborne bacterium that causes a moderately low number of infections per year (ca. 23,150 cases worldwide in 2010), but it is of major concern due to the high fatality rate in such cases (20–30%). The disease, named listeriosis, can manifest as a mild to severe gastroenteritis in most individuals, even with the ingestion of heavily contaminated food (up to 10^9 bacterial cells). However, in the cases of immunocompromised patients, pregnant women, children and the elderly, the ingestion of food even with a low number of

Published online: 08 June 2018

bacteria ($\sim 10^2$ to 10^4 cells) can lead to bacterial sepsis (Radoshevich and Cossart 2018). Controlling *Listeria monocytogenes* is a challenge for the food industry due to the bacterial arsenal of regulatory factors that confer the ability to oscillate between survival in the environment and survival in the human host (Radoshevich and Cossart 2018). Lactic acid bacteria (LAB) produce many antimicrobials with a long tradition of use in food preservation (Ndahetuye et al. 2012). Among the LAB-produced antimicrobials, there is great interest in bacteriocins, which are ribosomally encoded peptides that selectively kill phylogenetic relatives (Ndahetuye et al. 2012; Ghequire and De Mot 2018). Interestingly, there are many reports on bacteriocins produced by LAB with antilisterial activity (Fontanta et al. 2015). Moreover, many strains of *Lactobacillus* naturally colonize the intestine and can be used as probiotics to develop commercial products. Probiotics are defined as live microorganisms that when administered in adequate amounts, confer a health benefit to the host (FAO/WHO 2001; Hill et al. 2014). The FAO/WHO (2002) recommends the selection of probiotics by evaluating (i) gastrointestinal resistance, (ii) ability to hydrolyze bile salts, (iii) adhesion to intestinal epithelial cells, (iv) inhibition of adhesion of pathogens and (v) antimicrobial activity toward pathogenic bacteria. It is crucial to guarantee the viability of probiotic strains during their gastrointestinal transit, and their encapsulation in hydrocolloid beads may protect them from adverse conditions (Shah et al. 2016).

Lactobacillus curvatus CRL705, isolated from fermented artisanal sausage, produces lactocin 705 (a two-component peptide) and lactocin AL705 (an anti-*Listeria* peptide), which are active against other LAB, *Brochothrix thermosphacta* and different strains of *Listeria* (Castellano et al. 2001, 2003). *L. curvatus* CRL1532 and CRL1533 and *L. sakei* CRL1613 are also meat isolates that produce antimicrobial peptides, and these LAB survive at low temperatures and tolerate high salt concentrations (Fontana et al. 2005).

The aim of this study was to encapsulate *L. curvatus* CRL705, CRL1532, and CRL1533 and *L. sakei* CRL1613 to enhance bacterial survival during gastrointestinal exposition and evaluate the probiotic potential of these bacteriocinogenic strains, with a focus on the mitigation of *L. monocytogenes* by decreasing the bacterial adhesion to and invasion of eukaryotic cells.

Methods

Bacterial strains

Lactobacillus curvatus CRL705, CRL1532, and CRL1533 and *Lactobacillus sakei* CRL1613 were isolated from fermented sausages and meat (Fontana et al. 2005). *Listeria monocytogenes* FBUNT was provided by the Facultad de Bioquímica Química y Farmacia, UNT, Argentina.

Bacterial encapsulation

Lactobacillus curvatus CRL705, CRL1532, and CRL1533 and *L. sakei* CRL1613 were encapsulated using a mixture of calcium alginate solution and skim milk (0.5% fat) according to Ross et al. (2008) with minor modifications. Bacterial cultures grown in MRS broth were centrifuged at $1000\times g$ for 10 min, washed with sterile distilled water and resuspended in skim milk. Then, equal volumes of 9 g sodium alginate l^{-1} and 200 g skim milk bacterial suspension l^{-1} ($\sim 1 \times 10^{10}$ cfu/ml) were gently mixed together. This alginate mixture was introduced into a 5 ml sterile syringe (Terumo®, Leuven, Belgium) and extruded through a needle (27.5 G: 13 mm long × 0.4 mm diameter, Terumo®). The alginate mixture was aseptically dropped into a 0.1 M calcium chloride solution (hardening solution) over 30 min. Calcium alginate capsules were formed by the cross-linking of sodium alginate in the presence of calcium ions. Finally, calcium alginate capsules were rinsed twice with distilled water, obtaining a mean diameter of 2 mm. The encapsulation yield (EY) was calculated by dividing the viable count of disrupted capsules by the initial cell load (DePrisco et al. 2015). Each bacterial suspension in skim milk without an encapsulating medium served as a free cell control. The bacterial population was evaluated by plating on an MRS agar medium and incubating at 30 °C for 48 h.

Evaluation of free and encapsulated bacterial resistance after simulated gastrointestinal (SGI) transit

Bacterial viability after simulated gastric and intestinal exposition was evaluated according to a modified protocol by Abeijón Mukdsi et al. (2016). Briefly, artificial gastric fluid, pH 3, was adjusted with HCl

(100 mM). Gastric juice consisted of $NaHCO_3$ (45 mM), KCl (7 mM), NaCl (125 mM), and 3 g pepsin l^{-1} (Sigma-Aldrich). Simulated intestinal solution, pH 8, was adjusted with NaOH (5 mM). The intestinal juice composition was 3 g Oxgall l^{-1} (dehydrated fresh bile; Sigma-Aldrich) and 1 g pancreatin l^{-1} (ICN Biomedicals). In brief, 1 ml of bacterial suspension ($\sim 1 \times 10^{10}$ cfu/ml) was transferred to simulated gastric juice (5 ml) and incubated at 37 °C in a shaker (MaxQ 4450, ThermoFisher) for 2 h. Next, the cells were harvested by centrifugation (6500×*g*, 5 min), washed twice with sterilized saline solution (9 g NaCl l^{-1}) and re-incubated with agitation at 37 °C in simulated intestinal juice for 2 h. Additionally, 30 capsules were placed in simulated gastric juice and subsequently in intestinal solutions, as described above. Viable bacteria after gastric and gastrointestinal exposition were enumerated by decimal dilution and plating on MRS agar, followed by incubation at 30 °C for 48 h. All experiments were performed at least twice, and colony counts were expressed as the log10 of cfu/ml.

Bile salt hydrolase (BSH) activity

MRS agar plates supplemented with 5 g chenodeoxycholic acid l^{-1} (Sigma-Aldrich) and 0.37 g $CaCl_2$ l^{-1} (Vetec) were spotted with 10 µl of each overnight culture of *Lactobacillus* and incubated at 37 °C for 72 h under anaerobiosis. The ability to hydrolyze bile salts was evidenced by a precipitation zone around the inoculation spots (Franz et al. 2001).

Anti-listeria activity of *Lactobacillus* cell-free culture supernatant

To determine the antilisterial activity of *L. curvatus* isolates (CRL705, CRL1532, CRL1533) and *Lactobacillus sakei* CRL1613, the spot-on-lawn assay was used. Briefly, overnight cultures of LAB in MRS broth were centrifuged (6500×*g* for 10 min), and the cell free supernatants (CFSs) were heat treated (80 °C, 5 min); in addition, the pH was adjusted to 6.5 with NaOH (4 N) and filtered sterilized. Serial two-fold dilutions of supernatants (10 µl) were spotted onto BHI plates (7 g agar l^{-1}) previously inoculated with 50 µl of *L. monocytogenes* FBUNT. Cultures were incubated at 30 °C for 48 h, and bacteriocin activity was quantified based on Mayr-Harting et al. (1972) as arbitrary units per milliliter (AU/ml), defined as the reciprocal of the highest dilution that presented an inhibition zone. Aliquots of the CFSs were reserved for testing the ability of *L. monocytogenes* FBUNT to adhere to and invade Caco-2 cells.

Epithelial cell line and adhesion assays

Caco-2 cells (ATCC HBT37) were obtained from the Rio de Janeiro Cell Bank (UFRJ, Rio de Janeiro, Brazil) and prepared according to Gomes et al. (2012). The adhesion of LAB and *L. monocytogenes* to epithelial cells was evaluated separately and in the presence of CFSs. Briefly, overnight cultures of *L. curvatus* CRL705, CRL1532, and CRL1533; *L. sakei* CRL1613; and *L. monocytogenes* were harvested by centrifugation at 6500×g for 7 min and washed twice with sterilized phosphate-buffered saline (PBS). The pellets were resuspended in Dulbecco's Modified Eagle Medium (DMEM, Sigma-Aldrich) at final concentrations of $1x10^8$ cfu/ml for LAB and 10^7 cfu/ml for *L. monocytogenes*. Caco-2 cells were expanded in monolayers using 24-well plates with DMEM under 5% CO_2 at 37 °C. LAB and *Listeria monocytogenes* FBUNT cultures were inoculated on to the monolayers (250 µl per well) for individual adhesion assays and further incubation for 30 min. After incubation, non-adherent bacterial cells were eliminated by washing the eukaryotic cell monolayers twice with PBS. Cells with adherent bacteria were harvested with trypsin, centrifuged at 6500×g for 5 min and resuspended in PBS. Adherent LAB and *L. monocytogenes* cells were enumerated on MRS and BHI agar plates, respectively, and incubated for 24 h at 37 °C. Another set of Caco-2 cell monolayers were inoculated with the pathogen and DMEM in the presence of 50 and 90% (v/v) of CFSs and treated as described above. The adhesion capacity of LAB strains and *Listeria monocytogenes* was expressed as the number of adherent bacterial cells divided by the inoculum size (total number of bacteria added) multiplied by 100. The inhibition of adhesion of *L. monocytogenes* in the presence of CFSs was expressed in percent and defined as 100 (1 − T1/T2), where T1 and T2 correspond to the numbers of adherent *Listeria* cells (cfu/well) in the presence and absence of LAB CFSs, respectively (Moroni et al. 2006).

Influence of CFSs on *Listeria monocytogenes* invasion of epithelial cells

Listeria monocytogenes (10^7 cfu/ml) was inoculated in Caco-2 cell cultures as described in the previous section and incubated for 60 min at 37 °C in a 5% CO_2 atmosphere in the presence and absence of CFSs from the different lactobacilli (50 or 90%). To kill the noninternalized bacteria, the cells were washed twice with PBS and incubated (60 min, 37 °C under 5% CO_2) in 250 ml of DMEM with 250 mg of gentamicin per ml. The Caco-2 cell monolayer was washed with PBS and lysed with 0.1% (v/v) Triton X-100 (Sigma-Aldrich). The cell lysates were serially diluted in PBS and surface plated onto BHI agar (0.1 ml) to enumerate intracellular bacteria. The invasion efficiency (percentage) was calculated as the log of the number of intracellular bacterial cells (log cfu) divided by the log of the total number of bacteria inoculated, multiplied by 100 (Gomes et al. 2012). The inhibition of invasion was defined as $100\ (1 - T_C/T_L)$, where T_C and T_L are the numbers of invading *Listeria* cells (cfu/well) in the presence and absence of CFSs, respectively (Moroni et al. 2006).

Statistical analysis

The results are presented as the averages of three independent experiments with intra-assay triplicates, and data were subjected to an analysis of variance (ANOVA). Tukey's multiple comparisons test was applied to evaluate significant differences with a level of significance of 5%. Analyses were performed using the software Graph Pad Prisma 5, USA.

Results and discussion

Survival of free and encapsulated *Lactobacillus* spp. in SGI conditions

Figure 1 shows there was a significant difference ($p < 0.05$) between the free initial cell load and the population after 2 h under SGI conditions. Ross et al. (2008) showed that the viability of free probiotic acid lactic bacteria decreased by approximately 5 log cycles under SGI compared to that of the initial cell load, and similar results were reported by De Prisco et al. (2015) for free *Lactobacillus reuteri* DSM 17938.

In the present study, to minimize the loss of viability after SGI treatment, microorganisms were encapsulated into alginate beads. The encapsulation yield (EY) ranged from 85.39 to 98.92% (data not shown), and no significant differences were found among the four strains tested. Similar EY values were reported by Corbo et al. (2016) using 20 g alginate l^{-1} as the matrix for the encapsulation of lactic acid bacteria (92.9–96.8%). The EY obtained for the four LAB strains evaluated showed that the encapsulation parameters used in this study were suitable and a significant ($p < 0.05$), and a the probiotic cells achieved higher tolerance to the SGI conditions. Figure 1 shows that after the exposure of LAB to SGI conditions, the viable cell count from the capsules for all strains was higher than 9.30 cfu/ml. Alginate encapsulation allowed viable cells to reach an appropriate level for use as a probiotic, based on the usual effective dosage of 10^7–10^9 cfu/ml (Chandramouli et al. 2004). These results indicate that LAB encapsulation using alginate as a matrix makes lactobacilli safe and effective for oral delivery, supporting data obtained by other authors (Chandramouli et al. 2004; Ross et al. 2008).

Bile salt hydrolysis

All strains were able to hydrolyze bile salts, determined qualitatively by visual observation of precipitation zones around the inoculation spot (Fig. 2). This property is associated with the presence of conjugated bile salt hydrolase.

Adhesion of lactobacilli strains to Caco-2 cells

All *Lactobacillus* strains presented intermediate aggregative characteristics, showing an adhesion range from 63.6 to 68.6% (Table 1). Consistent with these results, other *Lactobacillus* strains reported in the literature adhered to cell lines, such as Caco-2, HT29 and HeLa 229 (Aazami et al. 2014). Cell adherence is directly related to the aggregation phenotype, which allows lactobacilli species to act as barriers to prevent the colonization of the intestine by pathogenic bacteria. The lactobacilli used in this study were previously shown to present intermediate aggregative characteristics based on the

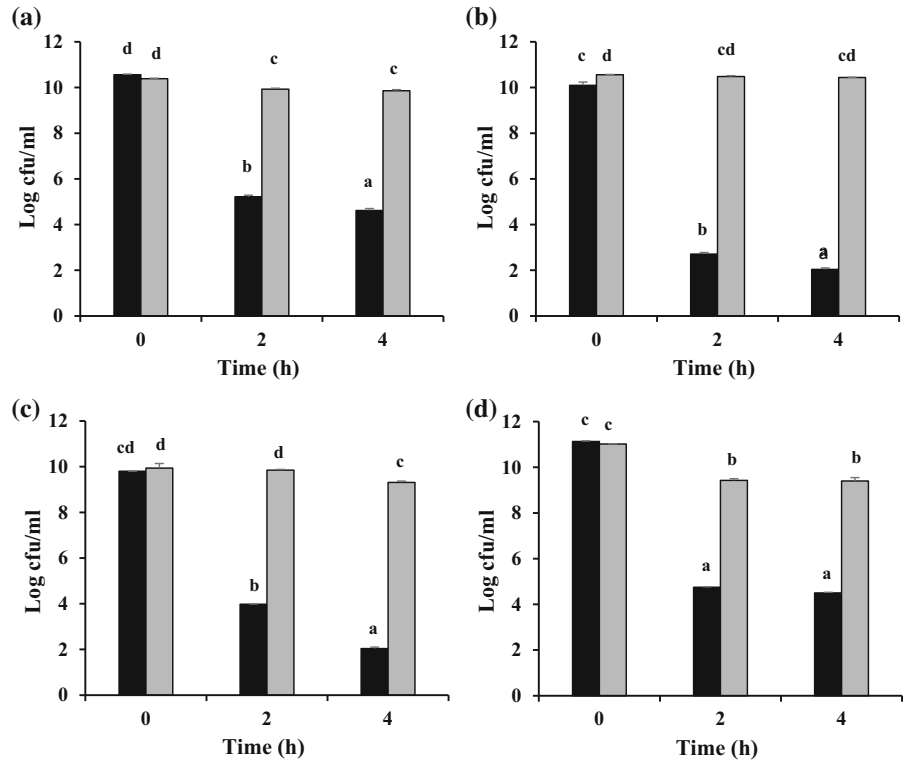

Fig. 1 Survival of (black filled square) free and (grey filled square) encapsulated *Lactobacillus* strains under SGI conditions. Each bar represents the average of triplicate counts from three independent trials, and error bars indicate the standard deviations of the three replicates. **a** *L. curvatus* CRL705, **b** *L. curvatus* CRL1532, **c** *L. curvatus* CRL1533 and **d** *L. sakei* CRL1613

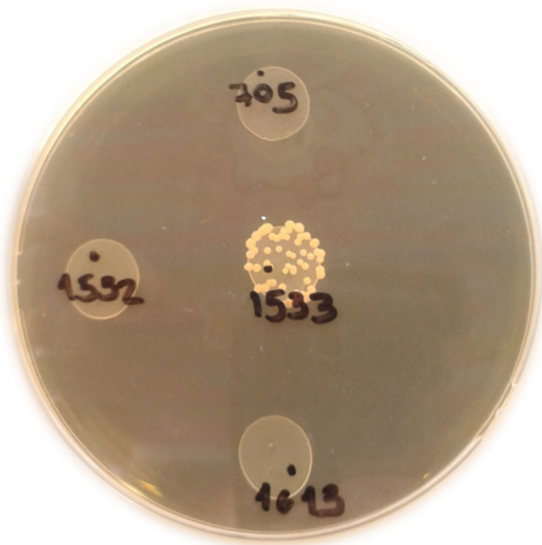

Fig. 2 Image of an agar plate showing the results of the bile salt hydrolysis test for *L. curvatus* CRL705, CRL1532, and CRL1533 and *L. sakei* CRL1613. MRS agar was used with 0.5% of chenodeoxycholic acid and 0.37 g $CaCl_2$ l^{-1}. Bile acid precipitates around the colonies (opaque halo) or the formation of opaque granular white colonies with a silvery shine were considered to indicate BSH activity

autoaggregation percentages, and they showed a biofilmogenic phenotype that was expressed under the shelter of mucus to confer epithelial cell protection (Pérez Ibarreche et al. 2014).

Anti-listeria activity of CFSs

The anti-listeria activities of the CFSs of *L. curvatus* CRL705, CRL1532, CRL1533 and 1613 in MRS broth were 3200, 1600, 800 and 800 AU/ml, respectively. CFSs were neutralized to rule out the effects of organic acids, indicating the presence of bacteriocin-like inhibitory substances as responsible for the antilisterial activity detected. Moreover, it was previously reported that *L. curvatus* CRL1532 and CRL1533 and *L. sakei* CRL1613 carried the *sppA*, *spp*Q and *sap*A structural genes, encoding for sakacin P, sakacin Q and curvacin A, respectively (Fontana et al. 2015). Additionally, for *L. curvatus* CRL705, a draft genome sequence revealed genes involved in bacteriocin production, including lactocin 705, sakacin P, sakacin Q, sakacin X, and sakacin T (Hebert et al. 2012).

Table 1 Adhesion of lactobacilli strains to Caco-2 cells

Strain	Initial cell load (log cfu/ml)	Bacteria adhered (log cfu/ml)	Adhesion (%)
L. curvatus CRL705	8.00 ± 0.20[a]	5.29 ± 0.26[a]	66.1[a]
L. curvatus CRL1532	8.30 ± 0.22[a]	5.60 ± 0.25[a]	67.5[a]
L. curvatus CRL1533	8.89 ± 0.25[b]	5.65 ± 0.16[a]	63.6[b]
L. sakei CRL1613	8.20 ± 0.19[a]	5.63 ± 0.09[a]	68.6[a]

Mean values and standard deviations of three replicates are presented. Percentage of adhesion: log cfu/ml bacteria adhered/log cfu/ml initial cell load × 100. Values with different letters within the same row differ significantly ($p < 0.05$)

Inhibition of *L. monocytogenes* adhesion to and invasion of Caco-2 cells

All CFSs were active against *L. monocytogenes* FBUNT at both concentrations tested (90 and 50%), corresponding to 2880 and 1600 AU/ml for *L. curvatus* CRL705; 1440 and 800 AU/ml for *L. curvatus* CRL1532; and 720 and 400 AU/ml for *L. curvatus* CRL1533 and *L. sakei* CRL1613, respectively. A small decrease in *L. monocytogenes* adhesion to Caco-2 cells at the highest concentration of all CFSs from the four lactobacilli strains was observed as illustrated in Fig. 3. Similarly, the bacteriocinogenic meat-derived strain *L. sakei* CTC494 counteracted the adhesion of *L. monocytogenes* to HT29 in competition experiments (Aazami et al. 2014). The anti-invasive effect of the CFSs from the four *Lactobacillus* strains tested on Caco-2 cells against *L. monocytogenes*

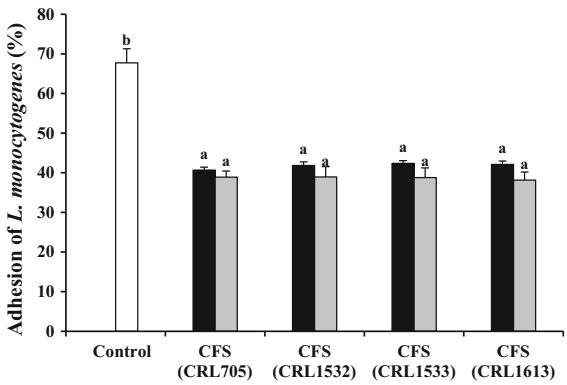

Fig. 3 Protective effect of CFSs from *L. curvatus* CRL705 (CRL705), *L. curvatus* CRL1532 (CRL1532), *L. curvatus* CRL1533 (CRL1533) and *L. sakei* CRL1613 (CRL1613) against the adhesion of *L. monocytogenes* to Caco-2 cells. Each bar represents the average of triplicate counts from three independent trials, and error bars are the standard deviations of three replicates. (black filled square) 50 and (grey filled square) 90% of CFSs. (open square) Control (without CFSs)

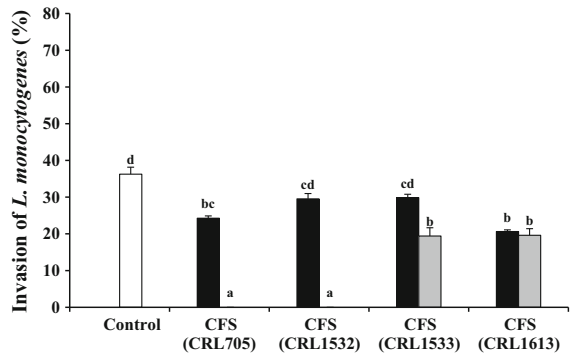

Fig. 4 Protective effect of CFSs from *L. curvatus* CRL705 (CRL705), *L. curvatus* CRL1532 (CRL1532), *L. curvatus* CRL1533 (CRL1533) and *L. sakei* CRL1613 (CRL1613) against the invasion of Caco-2 cells by *L. monocytogenes*. Each bar represents the average of triplicate counts from three independent trials, and error bars are the standard deviations of three replicates. (black filled square) 50 and (grey filled square) 90% of CFSs. (open square) Control (without CFSs)

FBUNT showed that all CFSs had a protective role against invasion by the pathogen (Fig. 4). *L. monocytogenes* was unable to invade Caco-2 cells when 90% CFSs from *L. curvatus* CRL705 and CRL1532 were added to the cultures ($p < 0.05$). The presence of the CFSs of *L. curvatus* CRL1533 and *L. sakei* CRL1613 significantly reduced the invasive capacity of the pathogen, with an invasion percentage of 19.42 and 19.61%, respectively. The CFSs at 50% from *L. curvatus* CRL1532 and CRL1533 showed a similar protective activity against invasion by the pathogen, whereas those of *L. curvatus* CRL705 and *L. sakei* CRL1613 were the most effective. *L. monocytogenes* FBUNT invasion of Caco-2 cells (36.25% ± 1.90) was comparable to the results reported by Gomes et al. (2012), who detected an invasion capacity of 37.91% of *L. monocytogenes* ATCC19115 in a similar in vitro model.

A significant reduction of infection caused by *L. monocytogenes* FBUNT on epithelial cells was observed when the cell monolayers were pre-coated with CFSs from *L. curvatus* CRL705, CRL1532, and CRL1533 and *L. sakei* CRL1613 for 1 h. CFSs from each *Lactobacillus* strain were responsible for reducing invasion of the pathogen strain. Although no experiments were performed in this study to specifically evaluate the anti-infective effects of bacteriocins, it is hypothesized that the antilisterial compounds present in CFSs induce the permeabilization of the pathogen cell membrane and affect its adhesion and subsequent listerial infection (Montville and Bruno 1994). In fact, it was recently demonstrated in a germ-free mice model that selected lactobacilli strains were able to attenuate infection by *L. monocytogenes* (Radoshevich and Cossart 2018).

The results of this study showed that the four lactobacilli isolates from meat and fermented sausage possess various in vitro probiotic properties. All strains hydrolyzed bile salts and adhered to Caco-2 cells. Moreover, all strains presented anti-listerial activity and protective activity against *L. monocytogenes* FBUNT, as shown by adhesion and invasion assays. *L. curvatus* CRL705, CRL1532, CRL1533 and *L. sakei* CRL1613 encapsulated in alginate showed a high tolerance to low pH. The encapsulation process used in this study significantly protected the strains against SGI conditions, demonstrating that the calcium alginate matrix acted as a barrier against gastric juice and intestinal conditions. In conclusion, the meat-isolated bacteriocinogenic LAB present biopreserving capacity and may be beneficial to consumer health due to their potential probiotic characteristics.

Acknowledgements We are grateful to The Bilateral Cooperation Program CONICET-FAPESP (2013/50498-5) for financial support of this study. We also thank Lucas Oliveira Sousa and Vanessa Maciel de Souza from FCFRP-USP for their excellent technical assistance with the cell line assays.

References

Aazami N, Jouzani GS, Khodaei Z, Meimandipour A, Safari M, Goudarzvand M (2014) Characterization of some potentially probiotic *Lactobacillus* strains isolated from Iranian native chickens. J Gen Appl Microbiol 60:215–221

Abeijón Mukdsi MC, Argañaraz Martínez E, Perez Chaia A, Medina RB (2016) Feruloyl esterase activity is influenced by bile, probiotic intestinal adhesion and milk fat. Benef Microbes 7:597–607

Castellano P, Farías ME, Holzapfel W, Vignolo G (2001) Sensitivity variations of *Listeria* strains to the bacteriocins lactocin 705, enterocin CRL35 and nisin. Biotechnol Lett 23:605–608

Castellano P, Raya R, Vignolo G (2003) Mode of action of lactocin 705, a two component bacteriocin from *Lactobacillus casei* CRL705. Int J Food Microbiol 85:35–43

Chandramouli V, Kailasapathy K, Peiris P, Jones M (2004) An improved method of microencapsulation and its evaluation to protect *Lactobacillus* spp. in simulated gastric conditions. J Microbiol Methods 56:27–35

Corbo MR, Bevilacqua A, Speranza B, Di Maggio B, Gallo M, Sinigaglia M (2016) Use of alginate beads as carriers for lactic acid bacteria in a structured system and preliminary validation in a meat product. Meat Sci 111:198–203

De Prisco A, Maresca D, Ongeng D, Mauriello G (2015) Microencapsulation by vibrating technology of the probiotic strain *Lactobacillus reuteri* DSM 17938 to enhance its survival in foods and in gastrointestinal environment. LWT Food Sci Technol 61:452–462

Fontana C, Vignolo G, Cocconcelli P (2005) PCR-DGGE analysis for the identification of microbial populations from Argentinean dry fermented sausages. J Microbiol Methods 63:254–263

Fontana C, Cocconcelli P, Vignolo G, Saavedra L (2015) Occurrence of antilisterial structural bacteriocins genes in meat borne lactic acid bacteria. Food Control 47:53–59

Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) (2001) Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Report of a Joint FAO/WHO working group in Córdoba, Argentina. http://www.who.int/foodsafety/publications/fs_management/probiotics/en/index.htm

Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) (2002) Guidelines for the evaluation of probiotics in food. Report of a Joint FAO/WHO working group in London Ontario, Canada. http://www.who.int/foodsafety/publications/fs_management/probiotics2/en/index.html

Franz CM, Specht P, Haberer P, Holzapfel W (2001) Bile salt hydrolase activity of enterococci isolated from food: screening and quantitative determination. J Food Prot 64:725–729

Ghequire MGK, De Mot R (2018) Turning over a new leaf: bacteriocins going green. Trends Microbiol 26:1–2

Gomes BC, Rodrigues MR, Winkelströter LK, Nomizo A, de Martinis ECP (2012) *In vitro* evaluation of the probiotic potential of bacteriocin producer *Lactobacillus sakei* 1. J Food Prot 75:1083–1089

Hebert EM, Saavedra L, Taranto MP, Mozzi F, Magni C, Nader ME, Font de Valdez G, Sesma F, Vignolo G, Raya RR (2012) Genome sequence of the bacteriocin-producing *Lactobacillus curvatus* strain CRL705. J Bacteriol 194:538–539

Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S, Calder PC, Sanders ME (2014) Expert consensus document. The International Scientific Association for Probiotics and

Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 11:506–514

Mayr-Harting A, Hedges AJ, Berkeley RCW (1972) Methods for studying bacteriocins. In: Norris JR, Ribbons DW (eds) Methods in microbiology. Academic Press, New York, pp 313–342

Montville TJ, Bruno ME (1994) Evidence that dissipation of proton motive force is a common mechanism of action for bacteriocins and other antimicrobial proteins. Int J Food Microbiol 24:53–74

Moroni O, Kheadr E, Boutin Y, Lacroix C, Isma F (2006) Inactivation of adhesion and invasion of food born *Listeria monocytogenes* by bacteriocin-producing *Bifidobacterium* strains of human origin. Appl Environ Microbiol 72:6894–6901

Ndahetuye JB, Koo OK, O'Bryan CA, Ricke SC, Crandall PG (2012) Role of lactic acid bacteria as a biosanitizer to prevent attachment of *Listeria monocytogenes* F6900 on deli slicer contact surfaces. J Food Prot 75:1429–1436

Pérez Ibarreche M, Castellano P, Vignolo G (2014) Evaluation of anti-*Listeria* meat borne *Lactobacillus* for biofilm formation on selected abiotic surfaces. Meat Sci 96:295–303

Radoshevich L, Cossart P (2018) *Listeria monocytogenes*: towards a complete picture of its physiology and pathogenesis. Nat Rev Microbiol 16:32–46

Ross GR, Gusils C, Gonzalez SN (2008) Microencapsulation of probiotic strains for swine feeding. Biol Pharm Bull 31:2121–2125

Shah A, Gani A, Ahmad M, Ashwar BA, Masoodi FA (2016) β-Glucan as an encapsulating agent: effect on probiotic survival in simulated gastrointestinal tract. Int J Biol Macromol 82:217–222