Meat Science 97 (2014) 475–479

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Sakacin Q produced by *Lactobacillus curvatus* ACU-1: Functionality characterization and antilisterial activity on cooked meat surface

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ARTICLE INFO

Article history:
Received 24 June 2013
Received in revised form 30 October 2013
Accepted 6 March 2014
Available online 13 March 2014

Keywords:
Meat
Biopreservation
Sakacin Q
Lactobacillus

ABSTRACT

This work was conducted to evaluate the antilisterial activity of sakacin Q produced by *Lactobacillus curvatus* ACU-1 on the surface of cooked pork meat. A genetic re-characterization of the producer strain and a study of the structural genes involved in bacteriocin production were carried out as complementary data. Studies indicated that the bacteriocin was not attached to the producer cells favoring pre-purifications steps. Bacteriocin effectiveness was not compromised by adsorption to meat and fat tissues. Several ways of dispensing the bacteriocin onto the meat surface, namely cell culture, cell free supernatant (CFS), a mixture of both and freeze-dried reconstituted CFS, were investigated. The use of the latter was the most effective one to control Listeria growth within studied systems. *L. curvatus* ACU-1 and its bacteriocin presented promising technological characteristics that made them suitable for meat biopreservation.

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1. Introduction

Currently, natural antimicrobials have become increasingly attractive for application in food products. Bacteriocins produced by lactic acid bacteria (LAB) can be considered as such. They are ribosomally-synthesized peptides or small proteins secreted into the microbial environment where they may cause the inhibition of spoilage bacteria and food-borne pathogens. Bacteriocin production takes place during exponential growth phase or at the end of it, keeping a close relationship with biomass production (Cleveland, Montville, Nes, & Chikindas, 2001). LAB bacteriocins are generally stable at acidic and neutral pH values showing well adaptation to the environment of the producer cells. Their chemical nature makes them prone to be involved in a number of food-related events, namely interaction with food components, precipitation, inactivation, or uneven distribution of bacteriocin molecules in the food matrix. Moreover, the efficacy of bacteriocins in foods will greatly depend on some other limiting factors such as the food microbiota and the target bacteria (Gálvez, Abriouel, Lucas López, & Ben Omar, 2007). Gänzle, Hertel, and Hammes (1996) stated that bacteriocin activity may be affected in food matrices by (i) changes in solubility and charge of the bacteriocins; (ii) binding of the bacteriocins to food components; (iii) inactivation by proteases, and (iv) changes in the cell envelope of the target organisms as a response to environmental factors. Furthermore, food processing conditions, storage temperature, pH, and bacteriocin instability to pH changes constitute environmental factors that play a crucial role on the effectiveness of bacteriocins.

The use of LAB as bioprotective cultures for meat products brings significant benefits since the effect on sensorial characteristics is considered to be hidden or not perceived during ripening and/or refrigeration due to the low carbohydrate content and the strong buffering capacity of meat. In this food matrix, LAB do not produce drastic changes on sensory characteristics compared to the changes that take place in milk or fermented vegetables. However, when bioprotective strains are to be used in meat or its derivatives, microbial cultures should keep their inhibitory activity at low temperatures and not modify significantly the pH of meat. While assessing a bacteriocinogenic culture for meat fermentation or biopreservation of cooked meat products the complex nature of meat or meat products is a key factor that needs to be addressed. Whether bacteriocin production is *in situ* or *ex situ*, many environmental parameters influence growth and metabolic production. Thus, the selection of a bacteriocinogenic strain should take into account its ability to grow and produce the bacteriocin *in situ*, the bacteriocin diffusion through the meat (Dicks, Mellett, & Hoffman, 2004), its adsorption to food components such as proteins and fats (Aasen et al., 2003), the influence of specific ingredients, namely sodium chloride and nitrite (Verluyten, Messens, & De Vuyst, 2003), and also those conditions that

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http://dx.doi.org/10.1016/j.meatsci.2014.03.003
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could destabilize the bacteriocin biological activity (Sarantinopoulos et al., 2002).

Several authors believe that the unsatisfactory effect of bacteriocin-producing strains *in situ* is due to hydrophobic interactions of the bacteriocins with fat (Blom, Katla, Nissen, & Holo, 2001; Chumchalova, Josephsen, & Plockova, 1998; Davies et al., 1999). As previously said, many factors in the food model system may interfere with bacteriocin activity. Sakacin A may adsorb to meat and fat particles and this may result in its inactivation (Schillinger, Kaya, & Lucke, 1991). Some bacteriocins, like nisin, have a stabilizing effect on the fat–water interface (Bani-Jaber, McGuire, Ayres, & Daeschel, 2000); their association with fat is readily reversible and does not prevent their antilisterial action. Other bacteriocins, like sakacin P, bind tightly to lipids in the food matrix (Aasen et al., 2003). They may remain trapped, unable to interact with the target pathogen (Kouakou et al., 2009).

The strain *Lactobacillus curvatus* ACU-1, isolated from artisanal dry sausages manufactured in Chaco (Argentina), produces a bacteriocin like inhibitory substance (BLIS) which is active against *Listeria innocua* ATCC 33090 and several strains of *Staphylococcus aureus* (Castro, Palavecino, Herman, Garro, & Campos, 2011). These authors found that bacterial growth and BLIS production kinetics showed a growth-associated production similar to most of bacteriocinogenic LAB strains. This inhibitory substance showed to be heat stable, effective after refrigerated storage and freeze/thaw cycles and even active against pathogens when produced under refrigeration at 3% NaCl concentration. Thereby, its application in meat products as a protective culture and/or as part of a starter culture seemed promising. This work was conducted in order to study the antilisterial activity of the BLIS produced *in situ* or *ex situ* by the strain *L. curvatus* ACU-1, so as to evaluate its potential use as a bioprotective culture on the surface of cooked pork meat. Moreover, a genetic re-characterization of the producer strain and a study of the structural genes involved in bacteriocin production were carried out as complementary data.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Two strains were used in this study: i) the bacteriocin-producing strain *L. curvatus* ACU-1, isolated from artisanal dry sausages manufactured in Chaco (Argentina) (Castro et al., 2011), and ii) the indicator microorganism *L. innocua* ATCC 33090 - in lieu of *Listeria monocytogenes*, because of their similar response to stress factors (Friedly et al., 2008). Both bacteria were maintained as frozen stocks at −30 °C in the suitable medium and were propagated twice in the appropriate culture media at 30 °C before use. *L. curvatus* was recovered in de Man, Rogosa and Sharpe broth (MRS, Biokar Diagnostics, France), while *L. innocua* was recovered in Brain Heart Infusion (BHI, Biokar Diagnostics, France).

2.2. Determination of antimicrobial activity

The determination of the antimicrobial activity of *L. curvatus* ACU-1 after each treatment was performed using an agar well diffusion assay (AWDA) (Schillinger & Lücke, 1989). Briefly, 15 ml of molten BHI agar (1% agar) inoculated with 30 μl of an overnight culture of the indicator microorganism was poured in Petri dishes. Wells (diameter: 5 mm; capacity: 20 μl) were formed by carving the agar with a cork borer. Afterwards, 20 μl of each system whose activity is to be measured were placed in each well. The plates were then incubated aerobically for 24 h at 30 °C and were subsequently examined for zones of inhibition. Inhibition was recorded as negative if no zone was observed around the agar well. Bacteriocin title was expressed by means of arbitrary units (AU) per ml, calculated as AU = (1000/v)/d; being v: volume seeded in the well and d: dilution (Kouakou et al., 2009). One AU is defined as the reciprocal of the highest dilution showing a clear zone of growth inhibition (Van Reenen, Dicks, & Chikindas, 1998).

2.3. Genetic identification by 16S rDNA amplification and sequencing

Strain was cultured overnight at 30 °C in MRS broth and then harvested by centrifugation at 10,000 *g*. Genomic DNA from the strain was extracted using a purification kit (Wizard, Genomic, Promega, Wisconsin) following manufacturer's recommendations. The 16S rRNA gene sequence (corresponding to positions 27-1492 in the *Escherichia coli* gene) was PCR amplified as described by DeLong (1992), using a DNA thermal cycler Mastercycler (Eppendorf, Hamburg, Germany). Sequencing on both strands of PCR-amplified fragments was performed using the dideoxy chain termination method by the commercial services of Macrogen Inc. (Seoul, Korea). The 16S rRNA homology searches against the NCBI database were carried out using BLAST program (Altschul, Gish, Miller, Myers, & Lipman, 1990). Sequence was deposited at the GenBank database under the accession number JX979220.

2.4. PCR screening for bacteriocins structural genes

The primers used for the amplification of curvacin A, sakacin P, and sakacin Q were those described by Cocolin and Rantsiou (2007) (Table 1). PCR amplification was performed in a Mastercycler (Eppendorf, Hamburg, Germany) in 20 μl of a mixture that contained 50 ng of DNA, 0.5 μM of each primer, 1.5 mM $MgCl_2$, 0.2 mM deoxynucleoside triphosphates, 1× PCR buffer and 1U of *Taq* DNA polymerase (Inbio-Highway, Argentina). For the amplification the thermocycler was programmed with the following conditions: initial denaturation at 95 °C for 5 min, 35 cycles of 95 °C for 1 min, 50 °C for 45 s, 72 °C for 1 min, final extension at 72 ºC for 7 min and cooled to 4 °C until analysis. Agarose gels (2% wt.vol^{-1}) were used to visualize the PCR products under UV light.

2.5. Adsorption to producer cells

Bacteriocin-producing cells were cultured at 30 °C for 18 h. The pH of the culture was adjusted to 6.0 with 1 M NaOH to allow maximal adsorption of the bacteriocin to the producer cells, according to the method described by Yang, Johnson, and Ray (1992). The cells were then harvested (12,000 × g, 15 min, 4 °C) and washed with sterile 0.1 M phosphate buffer (pH 6.5). The pellet was re-suspended in 10 ml of 100 mM NaCl (pH 2.0) and stirred slowly for 1 h at 4 °C. The suspension was then centrifuged (12,000 × g, 15 min, 4 °C), the cell-free supernatant (CFS) was adjusted to pH 7.0 with sterile 1 M NaOH and the bacteriocin activity was tested by means of the AWDA, as described previously.

2.6. Adsorption to fat and meat

Determination of the adsorption of the bacteriocin to fatty tissue and pork was performed according to the method described by Leroy and De Vuyst (2005). Bacteriocin-containing cell-free culture supernatant was prepared by inoculating 1 liter of ready-made MRS broth (Oxoid) with *L. curvatus* ACU-1 (1%, v/v), incubation overnight at 30 °C and removing

Table 1
Primers used in this study.

Primers	Sequence (5′–3′)	Reference
curA	(**F**) GTAAAAGAATTAAGTATGACA (**R**) TTACATTCCAGCTAAACCACT	Remiger, Ehrmann, and Vogel (1996)
sakP	(**F**) ATGGAAAAGTTTATTGAATTA (**R**) TTATTTATTCCAGCCAGCGTT	Remiger et al. (1996)
sakQ	(**F**) ATGCAAAATACAAAAGAACTAA (**R**)CGCTTGTTTAGAGACACCCGTT	Cocolin and Rantsiou (2007)

the cells by centrifugation (15,500 ×*g*, 20 min, 4 °C). Fat and meat pork were obtained from a local store and flamed to reduce the superficial contamination. Fat or meat particles (cubes bounded by squared faces of approximately 1 cm^2) were added to the supernatant (50 ml) and bacteriocin activity in the supernatant was measured over time. A control experiment was performed in the absence of meat and fat. The pieces of meat and fat were placed in Petri dishes with BHI agar inoculated with the indicator strain *L. innocua*, to check if the bacteriocin adsorbed to the tissue still exerted its antimicrobial activity.

2.7. Adsorption to target cells

Adsorption of bacteriocin to target cells was performed according to the method described by Yildirim, Avşar, and Yildirim (2002). The target strain (*L. innocua* ATCC33090) was grown overnight in BHI broth at 30 °C and centrifuged (8000 ×*g*, 25 min, 4 °C). Cells were washed twice with sterile 5 mM phosphate buffer (pH 6.5) and re-suspended in the same buffer to an optical density (OD) at 600 nm equal to 1.0. The pH was adjusted to 6.5 with sterile 0.1 M NaOH. The cell suspension was mixed with an equal volume of the systems: CFS *L. curvatus* ACU-1 (800 AU ml^{-1}, pH 6.5) and reconstituted lyophilized CFS tenfold concentrated (3200 AU ml^{-1}, pH 6.5). Then, they were incubated at 30 °C for 1 h. After removal of cells (8000 ×*g*, 25 min, 25 °C), the activity of unbound bacteriocin in the supernatant was determined by the AWDA as described before. All experiments were run in duplicate.

The percentage adsorption of bacteriocin to target cells was calculated according to the following formula:

$$\%\,\text{adsorption} = 100\text{-}\frac{\text{AU/ml}_1}{\text{AU/ml}_0} \times 100$$

AU/ml_1 refers to the bacteriocin activity after treatment; AU/ml_0 refers to the original (before treatment) activity.

2.8. Functionality of the bacteriocin on meat surface

A piece of beef (~1 kg) was cooked/sterilized at 121 °C for 15 min, cooled at room temperature in aseptic conditions and sliced with a sharp blade. Then, beef slices were immersed during 5 min in the following preparations (pH 6.5): A) Cell suspension of the producer strain (~1×10^3 colony forming units per milliliter (CFU ml^{-1})); B) CFS of the producer strain; C) Cell suspension (A) + CFS of the producer strain (B) (800 AU ml^{-1}); D) Freeze-dried CFS of the producer strain reconstituted in distilled water (3200 AU ml^{-1}). Afterwards, slices were immersed in a cell suspension of *L. innocua* ATCC 33090 (~1×10^3 CFU ml^{-1}) and they were packaged in sterile polyethylene bags (Whirl Pack, England). They were stored for 28 days at 4–5 °C. Three bags were retrieved every 7 days, and samples of microorganisms were collected by swabbing a delimited surface with the aid of a sterile calibrated aluminum frame. Serial dilutions were made and listerial counts were assessed on Palcam Agar (Biokar Diagnostics, Beauvais, France) while LAB counts were taken on MRS agar (Biokar Diagnostics). Bacterial counts were made in triplicate and were expressed as CFU per square centimeter (CFU cm^{-2}). Growth of either the producer strain or the indicator microorganism on slices was also checked (systems E and F, respectively). Control samples of un-inoculated sterilized slices of meat were tested on Plate Count Agar (Oxoid, England) in order to monitorize post-contamination throughout the experimental run (system G). Measurements of pH were taken from every sample with a surface probe attached to a pHmeter (Testo, Germany).

2.9. Statistical analyses

Each trial was repeated twice and each determination was done in triplicate. Statistical analysis of the data (analysis of variance $p_v < 0.05$ and Duncan's test) was performed with StatGraphics plus version 5.0 software (Manugistics Inc., USA).

3. Results and discussion

3.1. Genetic identification by 16S rDNA amplification and sequencing

In a previous work (Castro et al., 2011), the bacteriocin-producing strain studied herein was genotypically characterized as *L. curvatus/sakei* ACU-1 since the fragment of the rRNA gene amplified from the strain not only exhibited 100% homology with respect to *Lactobacillus sakei* ATCC 15578 (GenBank accession number AF429524) but also with respect to *L. curvatus* (accession number AY375292). In order to overcome the difficulty found in its identification, which has been reported by many authors (Champomier-Vergès, Chaillou, Cornet, & Zagorec, 2002; Koort, Vandamme, Schillinger, Holzapfel, & Björkroth, 2004; Vermeiren, Devlieghere, & Debevere, 2004), a phylogenetic analysis based on 16S rRNA gene sequence (1387 bp) was performed. The fragment of the 16S rRNA gene amplified from strain *L. curvatus/sakei* ACU-1 exhibited 99,3% homology with respect to *L. curvatus* type strain DSM 20019 (ATCC 25601). As a consequence, the bacteriocinogenic strain was named after *L. curvatus* ACU-1 and the entire sequence was deposited in the Gen Bank database under the accession number JX979220.

3.2. PCR screening for bacteriocin structural genes

In order to identify the gene involved in the bacteriocin synthesis, PCR amplifications were performed on *L. curvatus* ACU-1 genomic DNA using primer pairs based on *L. curvatus* bacteriocin structural genes detailed in Table 1. A PCR product of about 200 bp was obtained when specific primers for the detection of sakacin Q structural gene *spp*Q were used (Cocolin & Rantsiou, 2007). On the contrary, none of the curvacin A (*curA*) and sakacin P (*sppA*) structural genes could be amplified suggesting that the corresponding gene clusters were not present in *L. curvatus* ACU-1. According to Mathiesen, Huehne, Kroeckel, Axelsson, and Eijsink (2005), mature sakacin Q is a hydrophobic, cationic peptide consisting of 49 amino acids, and it has a calculated pI of 8.9. These are common features for class II bacteriocins. Sakacin Q does not contain conserved sequence motifs that are characteristic of class IIa bacteriocins, whereas the genetic organization of its gene and its expression in the absence of other peptides indicate that it is a one-peptide bacteriocin. Thus, sakacin Q seems to belong to class IIc of the bacteriocins. They have antibacterial activity against species of lactobacilli and certain food-borne pathogens such as *L. monocytogenes*. Environmental conditions, particularly the pH, will affect sakacin stability and efficacy. Application possibilities are predominantly in the field of sakacin-producing starter or co-cultures for meat fermentations since addition of purified sakacin is not (yet) accepted in food additives legislation (Naidu, 2000). Although production of sakacins is widely described in *L. sakei* strains, Cocolin and Rantsiou (2007) found that two strains of *L. curvatus* (L442 and LTH1174) were able to produce sakacins P and Q.

3.3. Adsorption of the bacteriocin to producer cells

Bacteriocin activity loss was detected after treatment with 100 mM NaCl pH = 2.0, suggesting that the bacteriocin was not attached to the producer cells. Several authors also reported this phenomenon for other bacteriocins [Ivanova, Kabadjova, Pantev, Danova, and Dousset (2000): bozacin B14; Todorov and Dicks (2005): pediocin ST18; Albano et al. (2007): pediocins HA-6111-2 and HA-5692-3; Pinto et al. (2009): bacteriocins bacALP7 and bacALP57, with similar molecular structure to enterocin B and pediocin PA-1, respectively]. This fact has remarkable technological connotations since it favors the pre-purifications steps of the antimicrobial peptides by ammonium sulfate precipitation (60% *w/v*) as suggested by Sambrook, Fritsch, and Maniatis (1989).

Table 2
Remaining bacteriocin activity in *L. curvatus* ACU-1 cell-free supernatant after adsorption to fat and meat tissues. Values express the percentage of bacteriocin activity retention.

Time (h)	Control	Fat	Meat
0	100	100	100
3	100	100	100
6	100	100	53
9	100	53	53
12	100	40	53
15	100	53	53
18	100	53	53
24	100	53	53

3.4. Adsorption to fat and meat tissues

This trial was conducted as a preliminary attempt to find out whether the bacteriocin sakacin Q released to the CFS by *L. curvatus* ACU-1 adsorbs to food matrices. Results are shown in Table 2. It can be observed that antimicrobial activity of sakacin Q had been halved in the presence of both food matrices (fat and meat tissues) which can be attributed to bacteriocin adsorption to them. Nevertheless, the remaining concentration, in both cases, was approximately 400 AU ml^{-1} at the end of the trial (after 24 h), being still effective against the target organism. Adsorption of the bacteriocin to fat and meat tissues appears to be the most probable explanation to this apparent decrease of the antimicrobial potency. A common characteristic of bacteriocins from LAB is their hydrophobicity, which could lead to an unspecified union of bacteriocin molecules to the hydrophobic surface of fat particles (Holzapfel, Geisen, & Schillinger, 1995). As an illustration, 88% loss of nisin activity was recorded by Jung, Bodyfelt, and Daeschel (1992) in the presence of 12.8% ($w.v^{-1}$) milk fat. In the same fashion, fat in a bulk of CFS from *L. sakei* CTC 494 caused a great loss of bacteriocin activity (Leroy & De Vuyst, 2005). Furthermore, Kouakou et al. (2009) found that high-fat content meat antagonizes the antilisterial effect of bacteriocinogenic *L. curvatus* CWBI-B28wt in pork meat systems. The latter trends could be explained by the study of Aasen et al. (2003) which showed that the activity of the bacteriocins in a medium depends not only on the fat content and type of bacteriocin, but also on the physical state of the medium (liquid or solid) and on whether the medium is shaken or not. As it can be presumed that part of the bacteriocin could exert its antimicrobial activity even if it is bound to the food matrix, a simple qualitative assay was performed by placing the small pieces of meat and fat, which were subjected to the aforementioned procedure, onto BHI agar seeded with *L. innocua*. Inhibition halos were recorded after incubation of the plates (data not shown). These preliminary results suggested that the adsorption of the bacteriocin to fat and meat tissues did not hinder its antimicrobial activity.

3.5. Antilisterial activity of the bacteriocin on meat surface

In order to evaluate the effectiveness of the bacteriocin sakacin Q produced by *L. curvatus* ACU-1 on meat surface against *L. innocua* ATCC 33090, slices of meat were immersed in different bacteriocin preparations (previously described). Listeria viable counts from the different treatments can be appreciated in Fig. 1. *L. curvatus* ACU-1 reached a population of 10.43 $\pm$ 0.05 log CFU cm^{-2} at the third week of storage, diminishing to 8.80 $\pm$ 0.17 CFU cm^{-2} at the end of the trial, showing a good adaptation to the meat surface environment. At the beginning, microbial counts from un-inoculated systems (G) were 1×10^2 CFU cm^{-2}, reached 1×10^4 CFU cm^{-2} at 21 days and ended up being 1×10^2 CFU cm^{-2}, suggesting that microbial population, other than the tested ones, was not numerous so as to condition the growth of the inoculated strains. *In situ* bacteriocin production (system A) significantly reduced listerial counts (approximately 5 Log cycles at the end of the trial). These results were expected and can be partially explained by 50% of bacteriocin sakacin Q adsorption to target cells, bringing a high antimicrobial effectiveness, regardless the food matrix. System B, which comprised the CFS of the bacteriocin-producing strain, also inhibited Listeria growth, but the reduction decreased after 3 weeks, being 3 log cycles higher than the one observed for *in situ* production. The joint presence of the producer cell and the CFS (system C) was statistically equally effective to the use of the producer cell (system A) by the end of storage. However, a more pronounced listericidal effect up to the third week was verified in comparison with the use of the producer cell (system A) or the CFS (system B) (Fig. 1). For this reason, it can be postulated that the combination of *in situ* and *ex situ* bacteriocin production could be a highly promising way of bacteriocin supply. This strategy is based in optimizing bacteriocin production by its inclusion as stimuli. It has been previously applied, in the case of *Lactobacillus plantarum* NC8, a starter strain used in Spanish-style green olive fermentations, and *Leuconostoc citreum* GJ7, a kimchi isolate, this occurs through the addition of specific adjunct strains that induce bacteriocin-production (Chang, Lee, & Chang, 2007; Maldonado-Barragán, Caballero-Guerrero, Lucena-Padros, & Ruiz-Barba, 2013).

The freeze-dried reconstituted CFS (system D) was the most effective one against Listeria on meat surface, reducing its population to undetectable levels. Taking into consideration bacteriocin concentration of this system (3200 AU ml^{-1}), it can be presumed that microbial inhibition was concentration-dependent as systems B and C (800 AU ml^{-1}

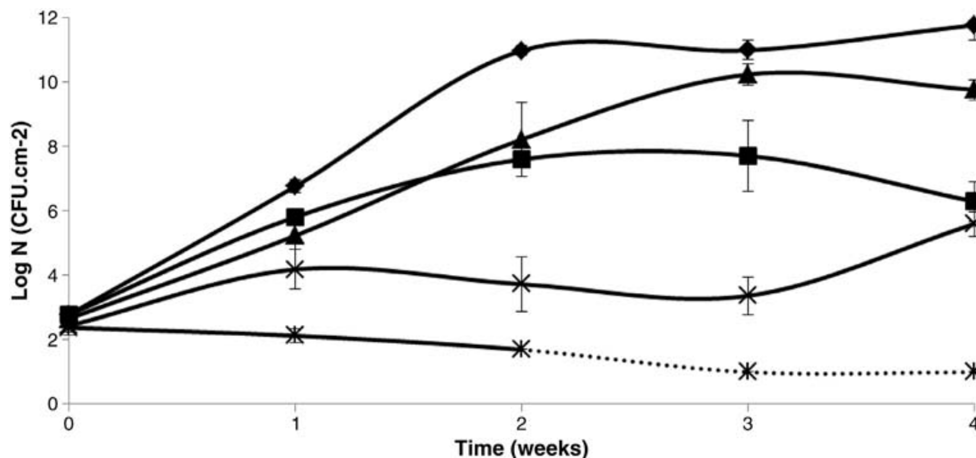

Fig. 1. Evolution of *Listeria innocua* ATCC 33090 count (expressed as Log N) on cooked meat surface systems: (◆) Listeria; (■) Listeria + *L.curvatus* ACU-1 cell culture; (▲) Listeria + cell-free supernatant (CFS); (x) Listeria + CFS + *L.curvatus* ACU-1 cell culture; (*) Listeria + freeze-dried CFS.

and presumably a higher concentration, respectively) had not exhibited such an extreme antimicrobial effect. This fact evidenced that bacteriocin adsorption to the food matrix might have been counteracted by the increase in bacteriocin concentration. Furthermore, there is still scarce information about active surface bacteriocin molecules and, consequently, it cannot be guaranteed that its adsorption fully endangers its inhibitory effectiveness against spoilage and pathogen microorganisms. It has to be highlighted that the pH values of the systems did not show statistically significant differences ($p > 0.05$) throughout the study. These values were within the range 5.43 ± 0.05 and 6.02 ± 0.05, meaning that pH decrease, because of LAB metabolic activity, was not responsible for the antilisterial effect observed throughout the study. This is in keeping with Kouakou et al. (2009) who tested antilisterial activity of bacteriocin-producing *L. curvatus* strain CWBI-B28 in lean pork meat co-cultured with *L. monocytogenes* at 4 °C.

4. Conclusions

The present work showed that *L. curvatus* ACU-1 has the ability to produce sakacin Q, and that this strain and the bacteriocin produced present technological characteristics that made them suitable for food applications. The bacteriocin was not attached to the producer cells favoring the pre-purifications steps. The adsorption of the studied bacteriocin to meat products main ingredients, namely meat and fat tissues, did not compromise its effectiveness. The use of the freeze-dried reconstituted CFS containing 3200 AU ml^{-1} was the most effective way to control Listeria growth within systems studied. Further study of sakacin Q *in situ* functionality either as food additive or being produced by starter, co- or protective *L. curvatus* ACU-1 cultures is needed if its potential for future use in meat preservation is to be exploited.

Acknowledgements

The authors acknowledge the financial support from Universidad de Buenos Aires, Universidad Nacional del Chaco Austral, Consejo Nacional de Investigaciones Científicas y Tecnológicas de la República Argentina and Agencia Nacional de Investigaciones Científicas y Tecnológicas de la República Argentina.

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