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Vol. 9(39), pp. 2095-2102, 30 September, 2015
DOI: 10.5897/AJMR2015.7575
Article Number: DF9DD5B55891
ISSN 1996-0808
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http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Growth and anti-listerial activity of a nisin Z producer in a pork lean meat broth fermentation system

Rosinéa Aparecida de Paula[1], Ana Andréa Teixeira Barbosa[1,2], Sílvia Regina Sartori Machado[1], Margarete Alice Fontes Saraiva[1], Célia Alencar de Moraes[1] and Hilário C. Mantovani[1]*

[1]Universidade Federal de Viçosa, Departamento de Microbiologia, Viçosa, Minas Gerais, 36570-900, Brazil.
[2]Universidade Federal de Sergipe, Departamento de Morfologia, São Cristóvão, Sergipe, Brazil.

Received 9 May, 2015; Accepted 6 July, 2015

***Listeria monocytogenes* is widely distributed in nature and has been isolated from numerous sources such as meat and fermented meat products. This pathogenic microorganism can resist conditions of low pH, low water activity (Aw), high salt (NaCl) concentrations and the presence of sodium nitrite, being able to survive the commercial sausage manufacturing process. The aim of this work was to evaluate the antilisterial activity of a lactic acid bacterium (*Lactococcus lactis* subsp. *lactis* PD 6.9) isolated from Italian-style salami in conditions that simulate salami fermentation. *L. lactis* PD 6.9 produces nisin Z and grows well in pork lean meat broth, a feature that would be useful to compete with food-borne pathogens. The peak of nisin Z production by *L. lactis* PD 6.9 in pork lean meat broth occurred after 14 h of fermentation, but the inhibitory activity decreased if the producer organism was maintained in stationary phase. When *L. lactis* PD 6.9 (10^7CFU ml^{-1}) and *Listeria monocytogenes* LMA 20 (10^6CFU ml^{-1}) were co-inoculated in pork lean meat broth, growth of *L. lactis* PD 6.9 was unaffected. The decrease in viable cell number of *Listeria* coincided with an increase in bacteriocin activity produced by *L. lactis* PD 6.9 in pork lean meat broth. Co-culture experiments indicated that *L. lactis* PD 6.9 was able to control the growth of *L. monocytogenes* even if the *Listeria* population was 1000-fold greater than the *L. lactis* population. These results demonstrate the potential application of *L. lactis* PD 6.9 in controlling the growth of *L. monocytogenes* during salami fermentation and its usefulness as a starter culture for fermented sausages.**

Keywords: *Listeria monocytogenes*, bacteriocins, lactic acid bacteria, co-cultivation, salami.

INTRODUCTION

Fermented sausages are produced by fermentation of minced meat mixed with fat, salt, curing agents (nitrate/nitrite), sugars and spices (Caplice and Fitzgerald, 1999). In order to speed the process and ensure the quality and uniformity of the final product, lactic acid bacteria (LAB) are commonly used as starter

*Corresponding author. E-mail: hcm6@ufv.br.Tel: + 55 (31) 3899-1946. Fax: + 55 (31) 3899-2573.

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cultures to decrease the pH (Marchesini et al.,1992; Liu et al., 2010). These LAB produce organic acids that enhance the aroma and extend the shelf life of the fermented product. However, the hurdles faced by microorganisms during sausage fermentation may not prevent the survival of pathogenic bacteria, including *Clostridium, Escherichia coli* O157:H7 and *Listeria* (Bonnet and Montville, 2005; Mor-Mur and Yuste, 2010; Martin et al., 2011; Hospital et al., 2012).

Listeria monocytogenes is a gram-positive food-borne pathogen widely distributed in nature and has been isolated from numerous sources such as meat and fermented meat products, including fermented sausages (Martin et al., 2011; Martins and Germano, 2011; Meloni et al., 2014). *L. monocytogenes* can resist conditions of low pH, low water activity (Aw), high salt (NaCl) concentrations and the presence of sodium nitrite, and is able to survive the commercial sausage manufacturing process (Bonnet and Montville, 2005; Degenhardt and Sant'Anna, 2007). The ingestion of food products contaminated with *L. monocytogenes* is particularly dangerous to young, old, pregnant and immune compromised individuals (Tauxe, 2002; Thévenot et al., 2005; Barlik et al., 2014) and even small number of *Listeria* appear capable to causing disease.

Bacteriocin-producing starter cultures appears to be an effective strategy to control *Listeria* in fermented sausages (Gormley et al., 2010; Freitas de Macedo et al., 2013; Rubio et al., 2014). *Lactococcus lactis* subsp. *lactis* PD 6.9, a lactic acid bacterium isolated from Italian salami (Maciel et al., 2003), was previously shown to inhibit the growth of *L. monocytogenes* in liquid culture and the antimicrobial activity was due to bacteriocin production (Carvalho et. al., 2006). Further work indicated that *L. lactis* PD 6.9 harbor the gene encoding nisin Z on the chromosome and purification of the peptide from the cell free supernatant followed by mass spectrometry analysis confirmed the molecular mass to be 3329.57 Da (Saraiva et al., 2014). Although *L. lactis* PD 6.9 showed great potential to inhibit foodborne pathogens, the production and activity of this bacteriocin had not yet been demonstrated in a liquid model system that simulates the conditions found during salami fermentation. Therefore, we hypothesized that *L. lactis* PD 6.9 could be useful as a starter culture for fermented sausages.

The aim of the present work was to examine the ability of *L. lactis* PD 6.9 to grow and produce bacteriocin in conditions that simulate the fermentation of minced meat and thus to verify its possible antagonism against *L. monocytogenes* in such medium.

MATERIALS AND METHODS

Microorganisms and growth conditions

L. lactis subsp. *lactis* PD 6.9, producer of nisin Z, was previously isolated from Italian salami processed by natural fermentation (Maciel et al., 2003), and kept stored at –80°C in D-MRS (Modified deMan, Rogosa e Sharpe media) (Hammes et al., 1992) supplemented with 20% glycerol. Before use, the culture was activated three times in D-MRS at 30°C.

L. monocytogenes LMA 20, isolated from chicken carcass, was obtained from the culture collection of the Food Microbiology Laboratory at the Universidade Federal de Viçosa, Viçosa, Minas Gerais State, Brazil (Carvalho et al., 2006). It was routinely transferred in tryptic soy broth supplemented with 0.6% yeast extract (TSBYE) and incubated at 37°C. Stock cultures were grown in the same medium and stored at –80°C.

Growth in a liquid model system that simulates the conditions found during minced meat fermentation

Portions of pork fresh lean meat (250 g) were obtained from a local abattoir, aseptically minced, placed into a blender (Walita Beta, São Paulo, Brazil) and mixed with approximately 200 ml of a 0.85% NaCl solution. The volume was adjusted to 500 mL and the mixture was centrifuged twice at 10 000 x *g* for 10 min at 5°C. The supernatant was collected and supplemented with 1.0% glucose, 3.0% NaCl, 120 ppm $NaNO_2$, 200 ppm $NaNO_3$. The pork lean meat broth was filtered through nitrocellulose membranes of 0.45 μm pore size (Schleicher and Schuell BioScience, Keene, USA) and stored at 4°C until use.

Cultures of *L. lactis* PD 6.9, were subcultured in pork lean meat broth (10^7 CFU ml^{-1}) and incubated at 25°C under aerobic conditions. Samples (100 µl) were taken at different time intervals up to 32 h, spread onto D-MRS agar plates and incubated at 30°C. Colony forming units per milliliter (CFU ml^{-1}) at each time interval were determined after 24 h of incubation. *L. monocytogenes* LMA 20 was inoculated into pork lean meat broth (10^5 to 10^6 CFU ml^{-1}), and incubated at 25°C under aerobic conditions. Growth was monitored by enumeration of the viable cell number for up to 32 h on tryptone soy agar supplemented with yeast extract (TSAYE) and incubated at 37°C. The experiments were performed at least in duplicate and the results represent the average from two independent observations. The specific growth rate (µ) of cultures was estimated from the rate of increase in cell number (X) based on the equation $dX/dt = \mu X$, where µ is an absolute rate constant with the units of h^{-1} and t is the growth time in exponential phase.

Antimicrobial activity in pork lean meat broth

L. lactis PD 6.9 was grown in pork lean meat broth to stationary phase. Inhibitory activity and pH were monitored over time. Aliquots (1 ml) of the cultures were centrifuged at 10 000 x *g* for 5 min, the supernatants were collected, and the pH was measured using an Accumet® model 15 pHmeter (Fisher Scientific, Pittsburg, USA). The supernatants were adjusted to pH 6.5 with NaOH 5 M, filtered in nitrocellulose membranes of 0.22 μmpore size (Schleicher and Schuell BioScience, Keene, USA) and tested for bacteriocin activity against *L. monocytogenes* LMA 20 by the agar well diffusion assay (Tagg et al., 1976).

Co-culture experiments in pork lean meat broth were carried out in batch cultures inoculated with 10^4 to 10^7 CFU $ml^{-1}$of *L. monocytogenes* LMA 20 and 10^1 to 10^7 CFU ml^{-1} of *L. lactis* PD 6.9, as indicated in the Figure legends. Samples of the co-cultures were taken at different time intervals and serially diluted (10-fold dilutions) into sterile saline solution (0.85% NaCl). Aliquots (20 µl) were plated onto D-MRS containing $CaCO_3$ (5 g l^{-1})and bromocresol purple (0.04 g l^{-1}) for viable counts of *L. lactis* PD 6.9. *L monocytogenes* was enumerated in TSAYE media supplement with 1.5% lithium chloride after 48 h of incubation. Culture pH was determined as described above. Co-culture experiments were also performed using a no-bacteriocinogenic strain, *Lactococcus lactis*

ATCC 19435. *L. monocytogenes* (10^7 CFU ml^{-1}) and *L. lactis* (10^7 CFU ml^{-1}) were co-inoculated in pork lean meat broth and the viable cell number was monitored up to 48 h as described above. The experiments were performed at least in duplicate and the results represent the mean from two independent observations. Enumeration of *L. monocytogenes* was determined from triplicate plate counts of each dilution.

Experimental design and statistics

The experiments were performed in two biological replicates. To evaluate the growth of *Listeria monocytogenes* and *Lactococcus lactis* PD 6.9 in pork lean meat broth, two samples for each time point were harvested for enumeration. To evaluate the antimicrobial activity pork lean meat broth, triplicate plate counts were prepared for each dilution and for each biological replicate. The error bars in figures indicate the standard deviation of the mean.

RESULTS AND DISCUSSION

Growth of *L. lactis* PD 6.9 and *L. monocytogenes* in pork lean meat broth

In this study, we tested the antagonistic properties of a nisin Z-producing strain of *L. lactis* that was previously isolated from naturally fermented Italian salami. When *L. lactis* PD 6.9 was inoculated (approximately 10^7 CFU ml^{-1}) in pork lean meat broth, the growth rate was 0.59 h^{-1} and the culture reached stationary phase after approximately 8 h of incubation (Figure 1a). The pH of the media decreased rapidly after 6 h of incubation and was as low as 4.2 at the end of the experiment. Bacteriocin activity was generally detected when the cell population was greater than 10^8 CFU ml^{-1}, and the maximum inhibitory activity occurred after 14 h of incubation (Figure 1b).

Bacteriocin purification and DNA sequencing demonstrated that the peptide produced by *L. lactis* PD 6.9 is a natural nisin A variant, nisin Z, as indicated by the substitution of a histidine by an asparagine residue at position 27 of the bacteriocin sequence (Saraiva et al., 2014). Nisin A and nisin Z appear to have similar biological activity, but often differ in their physicochemical properties, such as the diffusion is solid matrices.

In this study, the activity of nisin Z in the cell-free supernatants decreased after 14 h of fermentation, and inhibition zones could not be detected if cultures were maintained in stationary phase for more than 16 h. However, it should be noted that the same observation was done when the bacterium was cultivated alone in D-MRS media (data not shown). These results indicate that bacteriocin stability was not significantly affected by the formulation ingredients of the pork lean meat broth. The decrease in inhibitory activity could be explained by degradation or by adsorption to media constituents, but further experiments will be needed to clarify this point. Previous studies demonstrated that proteinase and peptidase activities associated with producer cells could be responsible for bacteriocin inactivation (Houlihan et al., 2004). Studies also showed that bacteriocins are less effective in food systems in which proteases are not inactivated (Vignolo et al., 1996; Castellano et al., 2004).

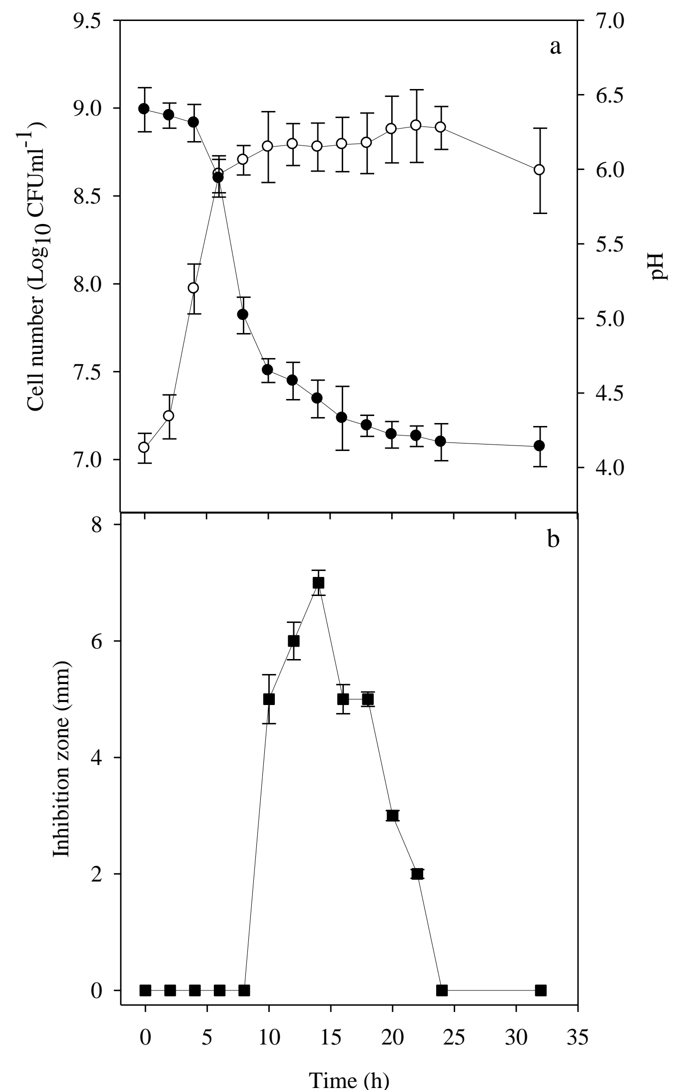

Figure 1. Growth (open circles) and medium pH (closed circles) of *L. lactis* PD 6.9 cultivated in pork lean meat broth at 25°C (a). Nisin Z activity (closed squares) presented as zones of inhibition caused by neutralized supernatants from *L.l actis* PD 6.9 against *L. monocytogenes* LMA 20 is shown in (b). The inoculum of *L. monocytogenes* LMA 20 used for the well diffusion assay was 10^6 CFU ml^{-1}.

Another mechanism for bacteriocin inactivation has also been reported by Rose et al. (1999). The authors used MALDI-TOF/MS to demonstrate that nisin added to fresh meat and meat juices was inactivated due to a reaction with glutathione (GSH), an abundant thiol compound found in animal tissues. GSH appears to react with multiples sites on a nisin molecule in a reaction

catalyzed by glutathione S-transferase (Rose et al., 2002). In our experiments, we used filtered-sterilized pork lean meat broth and the activity of nisin Z produced by *L. lactis* PD 6.9 was less than observed when the bacterium was grown in D-MRS media. These results suggest that a similar mechanism could affect the nisin Z activity of *L. lactis* PD 6.9 in raw meat. Additional experiments will be needed to test this hypothesis.

The fact that antagonistic activity reached its peak when the bacteriocin producer reached stationary phase suggest that *L. lactis* PD 6.9 could have an impact on the composition of the bacterial community that established during the early stages of the salami fermentation. Considering that salami fermentation and maturation can take as long as 30 days, *L. lactis* PD 6.9 could become a dominant culture during the salami manufacturing process. This hypothesis is supported by previous studies in which *L. lactis* PD 6.9 was isolated from salami after 6 days of fermentation and maintained its viability higher than 10^8CFU ml^{-1} even after 22 days of processing (Maciel et al., 2003).

The prevalence and the starter culture potential of *L. lactis* strain during salami fermentation was also showed by Cenci-Goga et al. (2008) and Frece et al. (2014). The authors demonstrated that *L. lactis* helped improving the sensory properties of the fermented product and enhanced the inhibition of pathogens such as *Listeria* spp and *Staphylococcus aureus* (Cenci-Goga et al. 2008; Frece et al., 2014).

If *L. monocytogenes* LMA 20 was inoculated (10^7 CFU ml^{-1}) into pork lean meat broth, growth occurred at a rate of 0.22 h^{-1} and stationary phase was reached after 15 h of cultivation (Figure 2). Processed meat products can provide an excellent environment for the growth of pathogenic organisms such as *L. monocytogenes* (Hereu et al., 2014; Heo et al., 2014). *L. monocytogenes*is a psychrotrophic and ubiquitous bacterium in meat products that is probably transferred from the environment to the food during processing (Mor-Mur and Yuste, 2010; Hereu et al., 2014). An early study performed by Martin et al. (2011) in small-scale factories producing traditional fermented sausage indicated that *L. monocytogenes* could be detected in equipments (11.8% of the samples), raw materials (28.9%), and even in the final products (15.8%). Because *Listeria* has shown resistance to various environmental stresses (heat, acidic pH, low water activity, low storage temperatures, etc.) commonly used in processed foods (Farber et al., 1993; Vogel et al., 2010) food industries have sought methods to control *Listeria* and ensure the safety of food products.

The addition of chemical additives such as nitrite to the meat can inhibit the growth of several pathogens and ensure the safety of the processed product. However, in this study, *L. monocytogenes* LMA 20 was able to grow in pork lean meat broth, even in the presence of NaCl and nitrite concentrations recommended for the fermented sausage manufacture process (Figure 2). This result could be explained in part by the higher water activity in the pork lean meat broth compared to the meat matrix of the fermented sausages, but growth of *Listeria* in model sausages added with salts and chemicals has also been observed (Benkerroum et al., 2003; Albano et al., 2007; Sansawat et al., 2013). Based on higher concentrations of salt and nitrite interfere with desirable characteristics of the processed product and nitrite represent risks to the consumer's health (by affecting the ability of hemoglobin to carry oxygen or producing carcinogenic nitrosamines), alternative methods are needed to prevent the growth of *Listeria* (Ananou et al., 2005; Ammor and Mayo, 2007; Freitas de Macedo et al., 2013).

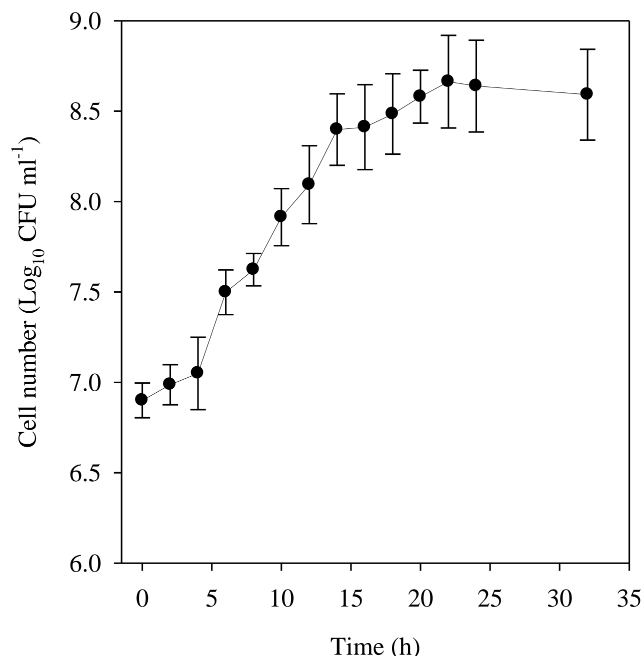

Figure 2. Growth of *L. monocytogenes* LMA 20 in pork lean meat broth at 25°C (open circles). A population of approximately 10^7 CFUml^{-1} of *L. monocytogenes* LMA 20 was used as the initial inoculum. Cell viability was monitored on TSAYE media after 48 h of incubation at 37ºC. Error bars show the standard deviation of the mean.

One such approach could be the use of bacteriocinogenic starter cultures to control potential food-borne pathogens. Bacteriocin are ribosomally-produced, extracellularly released antimicrobial peptides (post-translationally modified or not), which can have a relatively narrow spectrum of antibacterial activity (Deegan et al., 2006; Snyder et al., 2014). Bacteriocins are produced by many gram-positive and gram-negative bacteria and have been shown to inhibit the growth of several food-borne pathogens (Deegan et al., 2006; Snyder et al., 2014). Some bacteriocin-producing LAB have been isolated from fermented meat products and their ability to inhibit the growth of spoilage and pathoge-

nic microorganisms has been demonstrated (Albano et al., 2007; Liu et al., 2010; Snyder et al., 2014).

The potential application of bacteriocins in foods can be limited by properties such as spectrum of inhibition, heat stability and solubility. In general, the following should be considered when selecting bacteriocin-producing strains for food applications: 1) the producing strains should preferably be generally recognized as safe; 2) the peptide should be heat stable and have a broad spectrum of activity against pathogens such as *L. monocytogenes* and *Clostridium botulinum*; 3) the bacteriocinogenic strain and the antimicrobial peptide should pose no associated health risks and 4) the bacteriocinogenic strains should contribute to improve safety, quality and flavour of the food products (Rodríguez et al., 2002; Snyder et al., 2014). Considering that bacteriocin-producing bacteria are frequently isolated from several food sources, it appears that many of these bacteriocinogenic strains have been safely consumed for decades. Therefore, one could argue that the reintroduction of such cultures in a food system might have negligible negative impact on the safety of the consumers (Cleveland et al., 2001).

The main concern regarding a decreased efficiency of bacteriocins as biopreservatives is related with the emergence of nisin resistant strains, particularly in *L. monocytogenes* (Begley et al., 2010; Kaur et al., 2013; 2014). Kaur et al. (2013; 2014) demonstrated that nisin-resistant *L. monocytogenes* were selected after being exposed to high bacteriocin concentrations and nisin resistant strains did not become resistant to other preservation factors, such as low pH, sodium chloride, potassium sorbate or sodium nitrite. These authors also noted that nisin-resistant *L. monocytogenes* strains were generally more sensitive to food preservatives. Therefore, bacteriocins could be used as an additional hurdle to improve food safety without being undermined by resistance (Kaur et al., 2013). However, more studies are needed to determine the distribution of bacteriocin-resistant phenotypes among microorganisms that cause food spoilage and among food borne pathogens.

Co-culture of *L. lactis* PD 6.9 and *L. monocytogenes* in pork lean meat broth

When *L. lactis* PD 6.9 (10^7CFU ml^{-1}) and *L. monocytogenes* LMA 20 (10^6CFU ml^{-1}) were co-inoculated (10:1 ratio) into pork lean meat broth, growth of *L. lactis* PD 6.9 was unaffected and the specific growth rate was similar to that observed when the culture grew alone in pork lean meat broth (Figure 3). However, no increase in *L. monocytogenes* LMA 20 cell number was observed even after 12 h of incubation. Moreover, the decrease in *L. monocytogenes* LMA 20 cell number coincided with the production of nisin Z in the cell-free supernatants of *L. lactis* PD 6.9 cultured in pork lean meat broth (Figure 1b), and viability was approximately

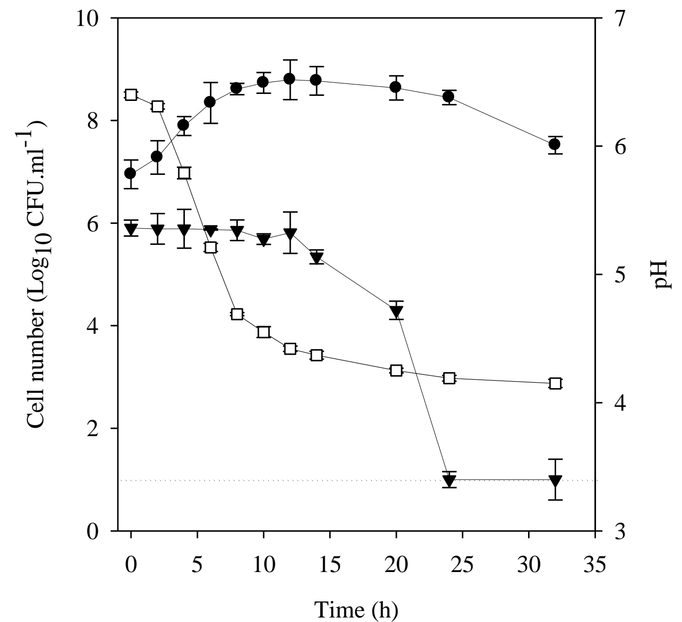

Figure 3. Co-culture of *L. lactis* PD 6.9 (closed circles) and *L. monocytogenes* LMA 20 (closed triangles) in pork lean meat broth at 25°C. Initial inoculums were 10^7CFU ml^{-1} and 10^6 CFUml^{-1} for *L. lactis* PD 6.9 and *L. monocytogenes* LMA 20, respectively. Medium pH (open squares) is also shown. The dotted line represents the detection level of the viable cell counts.

five log units lower after 25 h of co-cultivation (Figure 3).

Preliminary plating experiments showed that *L. lactis* PD 6.9 and *L. monocytogenes* could be unambiguously distinguished and enumerated if spread onto selective media (Figure 4). Co-culture experiments indicated that growth and bacteriocin production by *L. lactis* PD 6.9 was not affected in the presence of a target organism and the inhibitory activity could be inversely correlated with *L. monocytogenes* viability (Figures 1 and 3). *L. monocytogenes* cultivated in pork lean meat broth alone approached stationary phase after approximately 15 h of incubation. However, no growth was observed during the same period if *L. lactis* PD 6.9 was also added to the medium. These results suggest that *L. lactis* PD 6.9 could outgrow *L. monocytogenes* in conditions that simulate the salami fermentation process.

Because *L. monocytogenes* is tolerant to the hurdles of the salami manufacturing process, such as low pH and high osmolarity, the decrease in viable cell number could be explained by the sensitivity of the target organism to nisin Z. This idea was further supported by co-culture experiments using *L. lactis* ATCC 19435, a non-bacteriocinogenic *L. lactis* strain. When *L. lactis* ATCC 19435 (10^7CFU ml^{-1}) was co-inoculated into pork lean meat broth containing *L. monocytogenes* (10^7CFU ml^{-1}), the media pH decreased, but the viable cell number of *L. monocytogenes* did not change even after 48 h of

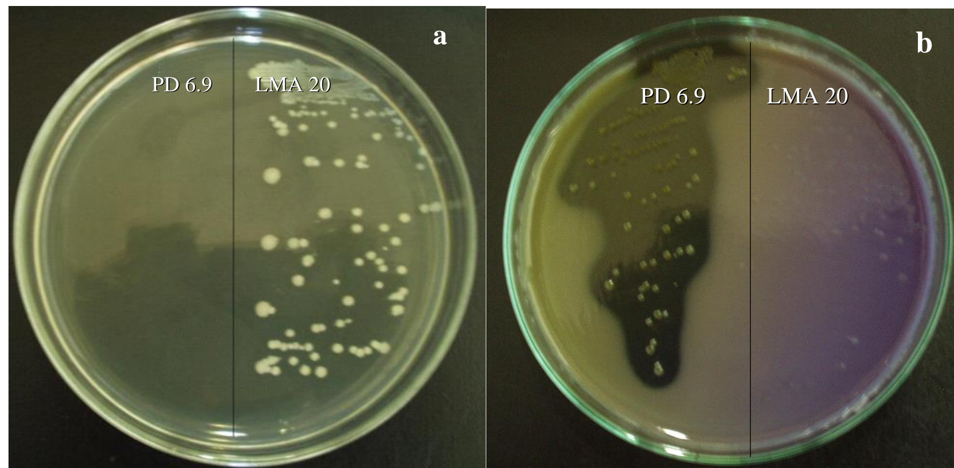

Figure 4. Selective media used for the enumeration of *L. lactis* PD 6.9 and *L. monocytogenes* LMA 20 in co-culture experiments. **a.** The growth of *L. monocytogenes* LMA 20 in TSAYE + 1.5% lithium chloride at 37°C. **b**. The growth of *L. lactis* PD 6.9 in D-MRS supplemented with $CaCO_3$ (5 g l^{-1}) and bromocresol purple (0.04 g l^{-1}) at 30°C. Both sides of the plates were spread with either *L. lactis* PD 6.9or *L. monocytogenes* LMA 20 before the plates were incubated.

incubation (data not shown). Because the cell number of *L. monocytogenes* LMA 20 did not change even when the pH was as low as 4.4 or in the presence of a non-bacteriocinogenic *L. lactis* strain, the bacterial antagonism appeared to be related with the production of nisin Z by *L. lactis* PD 6.9.

L. lactis PD 6.9 showed inhibitory activity against 10^7CFU ml^{-1} of *L. monocytogenes* LMA 20 even at low cell densities (Figure 5). When the cell number of *L. lactis* PD 6.9 inoculated in a co-culture varied from 10^1 to 10^7CFU ml^{-1}(1 million fold ratio variation between *L. monocytogenes* and *L. lactis* PD 6.9), the viability of *Listeria* was reduced 10 fold with a 10^3CFU ml^{-1}inoculum of *L. lactis* PD 6.9 (Figure 5). If the inoculum size increased to values equal to or greater than 10^5CFU ml^{-1}, *Listeria* counts were bellow detection level in the co-culture after 48 hours of incubation (Figure 5). When we tested a co-culture inoculated with a *Listeria* population more plausible to be found in contaminated foods (10^4CFU ml^{-1}), even 10^1CFU ml^{-1} of *L. lactis* PD 6.9 were able to reduce *L. monocytogenes* LMA 20 cell counts bellow the detection level (1 Log_{10} CFU ml^{-1}) in pork lean meat broth (not shown results).

L. monocytogenes is often found in raw meat at populations lower than 10^3 cfu/g (Buchanan et al., 1987; Thevenot et al., 2006), but our results indicate that even counts as high as 10^7CFU $ml^{-1}$of *L. monocytogenes* could be reduced bellow detection level after 48 hof incubation if *L. lactis* PD 6.9 was inoculated into pork lean meat broth at cell numbers greater than 10^4CFU ml^{-1}. Some food industries use starter cultures to ensure the safety and quality of fermented sausages and these cultures are commonly inoculated at 10^6 CFU g^{-1}. In conditions that approached the level of contamination normally found in

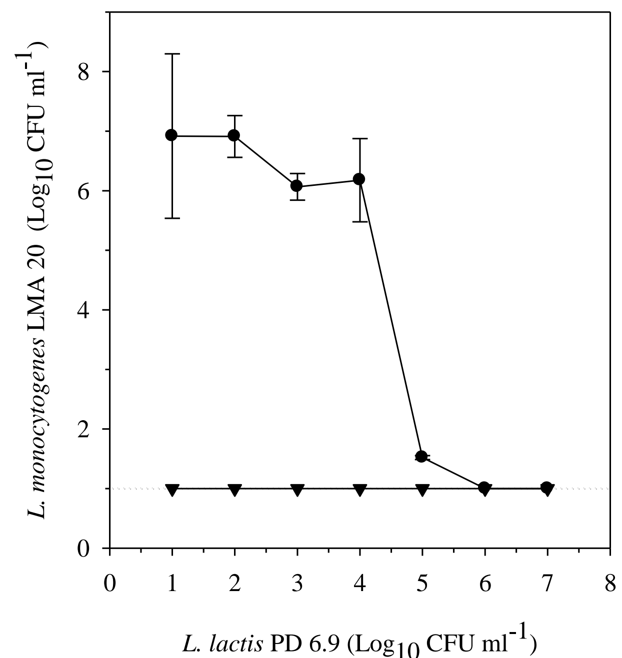

Figure 5. Co-culture of *L. monocytogenes* LMA 20 and *L. lactis* PD 6.9 in pork lean meat broth at various cell densities. Initial inoculum of *L. monocytogenes* LMA 20 was either 10^7 (closed circles) or 10^4 CFU ml^{-1} (closed triangles). The initial inoculum of *L. lactis* PD 6.9 varied from 10^1 to 10^7 CFU ml^{-1}. Viable cell numbers represent counts after 48 h of incubation at 37°C. The dotted line represents the detection level of the viable cell counts. Error bars show the standard deviation.

foods, *L. lactis* PD 6.9 could prevent the growth of *L.*

monocytogenes even if co-inoculated with only 10^1CFU ml^{-1}(1000:1 ratio between *L. monocytogenes* and *L. lactis* PD 6.9). Although the effect of *L. lactis* PD 6.9 against other important food-borne pathogens has not yet been assessed, this bacterium could be a potentially useful starter culture in salami fermentation. Our preliminary results indicate that at least some *Staphylococcus aureus* strains are also inhibited by *L. lactis* PD 6.9. Further studies will address if *L. lactis* PD 6.9 also inhibits starter cultures that are used for salami fermentation or interfere with the organoleptic characteristics of product.

Although our results were obtained in a model system (liquid model) that differs in composition from traditional technological processes for sausage production, the main conditions prevailing during salami fermentation that interfere with bacterial growth were maintained. It is well know that many bacteriocins behave differently in liquid and solid matrices, but the antimicrobial activity of nisin has been demonstrated in real sausages (Hampikyan and Ugur, 2007).

These results indicate that *L. monocytogenes* is inhibited in conditions that prevail during salami fermentation when co-cultured with *L. lactis* PD 6.9. The antagonistic and competitive properties of *L. lactis* PD 6.9 are relevant for its application as a starter culture for fermented sausages, even though the bacteriocin activity might be reduced by components of the salami system. Additionally, because *L .lactis* has been generally recognized as safe and the *L. lactis* PD 6.9 was obtained from a food source (Italian salami), its reintroduction in fermented salami should not impose toxicological problems for consumption of the final product. Furthermore, the use of bacteriocin-producing starter cultures is especially attractive to replace chemical additives or add new hurdles that are effective inhibiting the growth of spoilage and pathogenic bacteria during food processing.

Conflict of interests

The authors did not declare any conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Brazil. R.A. de Paula received a doctoral fellowship from the Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG).

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