Food Control 22 (2011) 954–958

Contents lists available at ScienceDirect

Food Control

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

Occurrence of *Listeria* spp. in Brazilian fresh sausage and control of *Listeria monocytogenes* using bacteriophage P100

Lívia P.R. Rossi [a], Rogeria C.C. Almeida [b,*], Layse S. Lopes [b], Ana C.L. Figueiredo [b], Maria P.P. Ramos [c], Paulo F. Almeida [d]

[a] Instituto Federal de Educação, Ciência e Tecnologia Baiano,Campus Catu. Rua Barão de Camaçari, 118, Barão de Camaçari, CEP: 48110-000. Catu- BA, Brazil
[b] Universidade Federal da Bahia, Departamento de Ciência dos Alimentos da Escola de Nutrição, Av. Araújo Pinho, 32, Canela, CEP: 40110-160. Salvador-BA, Brazil
[c] Universidade Federal do Espírito Santo, Departamento de Ciências da Saúde, Biológicas e Agrárias. Centro Universitário Norte do Espírito Santo. Rodovia BR 101 Norte, km 160, Litorâneo. CEP: 29932-540. São Mateus-ES, Brazil
[d] Universidade Federal da Bahia, Departamento de Ciências da Biointeração do Instituto de Ciências da Saúde, Avenida Reitor Miguel Calmon, 27, Canela, CEP: 40110-100. Salvador-BA, Brazil

ARTICLE INFO

Article history:
Received 31 August 2010
Received in revised form
23 November 2010
Accepted 4 December 2010

Keywords:
Listeria spp.
Bacteriophage P100
Control
Brazilian fresh sausage

ABSTRACT

Since the 1980s, an increase in outbreaks of human listeriosis linked to contaminated food has been a concern of health authorities. Intensively manipulated foods, such as Brazilian fresh sausage, are frequently responsible for food-borne diseases. In this work the occurrence of *Listeria monocytogenes* and the efficacy of bacteriophage P100 (LISTEX™) to control the microorganism was evaluated in Brazilian fresh sausage. Eighty samples were analyzed, 40 each of swine and chicken Brazilian fresh sausage. *Listeria* spp. were isolated from 12 samples (15%), of which three (3.75%) were positive for *L. monocytogenes*. *L. monocytogenes* strains isolated belonged to serotype 1/2a. *L. monocytogenes* 1/2a was inoculated in Brazilian fresh sausage (2.1×10^4 cfu/g) with the bacteriophage added thereafter (3.0×10^7 pfu/g). Samples were analysed immediately (day zero) and then stored at 4 °C for 10 days. The bacteriophage P100 reduced *L. monocytogenes* counts by 2.5 log units at both 0 and 10 days compared to controls without bacteriophage. In spite of this, the populations of *L. moncytogenes* increased over the 10 day storage. Our data demonstrate that in one of the samples the use of the bacteriophage dropped the bacteria count below the level of direct detection. This study demonstrates a new alternative for pathogen control in the food industry, especially in the processes used to produce Brazilian fresh sausage.
© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

Brazilian fresh sausage ("linguiça") is a mixture of minced pork and/or minced chicken, curing salts and spices, in a natural gut casing. This product is very popular in Brazil and is frequently consumed under-cooked.

In Brazil, food-borne listeriosis outbreaks have not yet been documented, but in recent studies, the presence of *Listeria monocytogenes* has been described in several products including Brazilian fresh sausage (Lima, Rossini, & Pompermayer, 2003; Silva et al., 2004; Sousa, Figueiredo, Maia, & Frizzo, 2006). Research conducted in Brazil reported an incidence of 100.0% of the *Listeria* spp. in raw meat used to make the product and the strain of *L. monocytogenes* was isolated in 16.6% of the final product (Silva et al., 2004).

Over the last few years, despite the recent technological advancements in pathogen control in foods, consumers have sought natural foods, i.e., foods submitted to less aggressive treatments without chemical preservatives. Moreover, the extensive use of sanitizers has led to the development of resistant bacteria, rendering these procedures less effective (García, Martínez, Obeso, & Rodríguez, 2008). In recent years, it has become widely recognized that bacteriophages have several potential applications in the food industry. Bacteriophages are naturally occurring viruses that are suitable candidates for the environmentally friendly biocontrol of pathogens (Guenther, Huwyler, Richard, & Loessner, 2009). They have been proposed as an alternative to antibiotics in animal health, as biopreservatives in food, and as tools for detecting pathogenic bacteria throughout the food chain (Atterbury, Connerton, Dodd, Rees, & Connerton, 2003; Leverentz et al., 2001). They are safe to use as they are harmless to mammalian cells, and their high host specificity allows proper starter performance in fermented products and keeps the natural microbiota

* Corresponding author. Tel./fax: +55 7132352416.
E-mail address: rogeriac@ufba.br (R.C.C. Almeida).

0956-7135/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodcont.2010.12.001

undisturbed (García et al., 2008). The attributes of bacteriophages include the following: (i) They kill bacterial target cells; (ii) They generally do not cross species or genus boundaries, and will therefore not affect (a) the desired bacteria in foods (e.g., starter cultures); (b) commensals in the gastrointestinal tract, or (c) the accompanying bacterial flora in the environment; (iii) Bacteriophages are generally composed entirely of proteins and nucleic acids, therefore their breakdown products consist exclusively of amino acids and nucleic acids (Carlton, Noordman, Biswas, Meester, & Loessner, 2005). Thus, they are not xenobiotics, and, unlike antibiotics and antiseptic agents, their introduction into and distribution within a given environment may be seen as an extension of a natural process (Carlton et al., 2005). Recently the USFDA announced that it had approved the use of a bacteriophage preparation made from six individually purified bacteriophages to be used on ready-to-eat meat and poultry products as an antimicrobial agent against *L. monocytogenes* (EBI Food Safety, 2007). The approved bacteriophage preparation is reported to be effective against 170 strains of *L. monocytogenes* (Lang, 2006). The commercial product named LISTEX™ P100 was approved as a food biopreservative and granted GRAS (Generally Recognized as Safe) status (FDA, 2006). This study was undertaken to investigate the presence of *Listeria* spp. in Brazilian fresh sausage and to investigate the efficacy of P100 in reducing populations of *L. monocytogenes* on laboratory-inoculated Brazilian fresh sausage.

2. Materials and methods

2.1. Investigation of Listeria spp. in Brazilian fresh sausage

2.1.1. Sampling and laboratory procedures

Eighty samples, 40 of swine and 40 of chicken Brazilian fresh sausage, were purchased at a local supermarket in Salvador, BA, Brazil. Portions of 250 g of food were aseptically collected between March and October of 2008 and samples were transported from the place of collection to the laboratory in an insulated cold box filled with ice. To detect bacteria in the samples the standard method of the United States Department of Agriculture (USDA)/Food Safety and Inspection Service (FSIS) (USDA/FSIS, 2005, chap. 8.04) was used. One *L. monocytogenes* positive control (*L. monocytogenes* Scott A, a serotype 4b, ATCC 15313) and one uninoculated media negative control were used for each set of concurrently analyzed samples. After aseptically removing the casing in a class II biosafety cabinet (Labconco model 36210 class BII, Brazil), samples of 25 g were blended with 225 ml of Modified University of Vermont broth (UVM, Difco Code No 022317) in a Stomacher (ITR model 1204, serie126, Brazil, 240 bpm) for two min and incubated for 24 h at 30 °C. Secondary enrichment was performed in Fraser broth (FB, Difco Code No. 211767) at 35 °C for 24–48 h. If any degree of FB darkening was evident (esculin hydrolysis), a volume of 100 μl of the broth was streaked onto Modified Oxford Listeria Selective Agar (MOX, Difco Code No. 222530), supplemented with moxalactam 20 mg/l and colistin sulfate 10 mg/l. Plates were incubated at 35 °C for 24–48 h. After that, at least 20 suspect colonies, if available, were streaked on 5% horse blood agar (blood agar base II, Difco Code No. 0045/17) plates and incubated at 35 °C for 24 h. After incubation, plates were examined for colonies surrounded by a small zone of β-hemolysis. Typical colonies were transferred to tryptic soy broth (TSB, Difco Code No. 211825) supplemented with 0.6% (w/v) yeast extract (Difco Code No. 211929) and confirmatory tests were carried out for Gram stain, catalase activity and motility in semi-solid indol motility medium (SIM, Difco Code No. 211578) at 25 °C for seven days, for the typical umbrella shape. For biochemical confirmatory tests, *Listeria* API (BioMerieux® S.A., Marcy L'Etoile, France) was used. Serological slide agglutination tests were done according to Seeliger and Hohne (1979) on all isolates presumed to be *Listeria*, using commercially prepared antisera (Difco).

2.2. Control of L. monocytogenes inoculated in Brazilian fresh sausage using bacteriophage P100

2.2.1. Bacterial strains, bacteriophage, media and culture conditions

The bacteria and bacteriophage used in this study were: a single strain of *L. monocytogenes* 1/2a isolated from Brazilian fresh sausage; *Listeria ivanovii* WSLC 3009 (SLCC 4769); and bacteriophage P100 (LISTEX™ P100) provided by EBI Food Safety (Wageningen, The Netherlands).

L. ivanovii was used as helper strain for the P100 bacteriophage (Carlton et al., 2005; Loessner & Busse, 1990). The culture strains were stored in a Hogness medium (1.3 mM $K_2HPO_4 \cdot 3H_2O$; 1.3 mM KH_2PO_4; 2.0 mM citrate-Na$\cdot 2H_2O$; 1.0 mM $MgSO_4 \cdot 7H_2O$; 4.4 % (v/v) glycerol) and frozen at −80 °C. Before use, the *L. monocytogenes* culture was activated in tryptic soy broth supplemented with 0.5% (w/v) yeast extract (TSB-YE) at 35 °C overnight in a shaker (Cientec model CT 712, Brazil) at 150 rev/min. The *L. ivanovii* culture was grown overnight at 30 °C in a half-concentrated brain-heart infusion broth (BHI, ½ v/v, Difco Code No. 237500) with the NaCl concentration adjusted to 5 g/l.

In all experiments, the top layer agar (semi-soft agar or overlay agar) was prepared by adding 0.4% (w/v) agar to BHI. To improve the bacteriophage plaques, 0.75% (w/v) glycine (Sigma Aldrich - Poole, United Kingdom) was added to the top layer agar (Lillehaug, 1997). Appropriate bacterial dilutions were made in lambda buffer (6 mmol/l Tris buffer, pH 7.2; 10 mmol/l Mg $(SO_4)_2 \cdot 7H_2O$; 50 μg/ml gelatin).

Bacterial survival following treatment with the P100 phage was determined by measuring colony-forming units (cfu/g) on Modified Oxford Listeria Selective Agar (MOX) supplemented with moxalactam 20 mg/l and colistin sulfate 10 mg/l.

2.2.2. Titration of P100 bacteriophage

The titer of the P100 was determined according to a protocol suggested by EBI Food Safety (personal communication). This consisted of serial dilutions of the bacteriophage suspension in a lambda buffer, followed by the incorporation of 100 μl into 3.5 ml of the molten overlay agar cooled to 45 °C, which contained 150 μl of *L. ivanovii* culture grown overnight at 30 °C in a ½ strength BHI. This was poured onto BHI agar (1.2% w/v agar) plates and incubated at 30 °C for 20–24 h. Plaques were counted and the titer was determined as plaque-forming units (pfu/ml).

To recover the bacteriophage from food without *L. monocytogenes* (phage control), the sample was diluted in lambda buffer and an aliquot of 100 μl was incorporated into 3.5 ml of the molten overlay agar cooled to 45 °C, which contained 150 μl of *L. ivanovii* (helper strain). As mentioned above, the mixture was poured onto BHI agar (1.2% w/v agar) plates and incubated at 30 °C for 20–24 h. Plaques were counted and the titer was determined as plaque-forming units (pfu/g).

2.2.3. Preparation of bacteria inoculum

L. monocytogenes 1/2a was subcultured at least twice by loop inoculation of 10-ml volumes of tryptic soy broth containing 0.5% (w/v) yeast extract (TSB-YE), which was then incubated at 35 °C for 18–20 h in a shaker at 150 rev/min. The cell suspensions were transferred to sterile eppendorf tubes and inoculum levels were confirmed by surface plating duplicate samples on MOX. The plates were incubated at 37 °C for 24 h before colony counts were obtained. This experiment was repeated three times in duplicate (Valadares, 2000). Cell suspensions were diluted in an appropriate amount of 0.1% (w/v) peptone water to give a cell number of 10^5 cfu/ml and were used immediately for sample inoculation.

Download English Version:

https://daneshyari.com/en/article/6394642

Download Persian Version:

https://daneshyari.com/article/6394642

Daneshyari.com