In-vitro experiment of Listeria reduction in ready-to-eat dry cured sausages

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Abstract

The risk of listeriosis associated with ready-to-eat foods is a major concern in United States. The recently published United States regulations require ready-to-eat meat producers to control Listeria monocytogenes, using interventions which may include antimicrobials that reduce post-processing contamination by at least 1 log cycle and that no more than 1 log increase throughout product shelf life. This regulation impact also the Spanish meat producers especially dry cured sausages, which export their products to USA. In this study, we analyzed in vitro, individually and in combinations, the commonly applied antimicrobials to reduce Listeria. Performing in-vitro experiment before applying directly on dry cured sausages offer us the benefits such as time and cost saving. Optimum concentration of each treatment was firstly determined, which is the concentration that kills 50% of population of the two strains used, Listeria monocytogenes and its surrogate, Listeria innnocua (105CFU/ml). The results showed that there are two different affects of treatments, bactericidal effect (obtained with bacteriophage, nisin, and pediocin-producing Pediococcus acidilactici as additives) and bacteriostatic effect (obtained with organic acids, lyzozyme and lactic acid bacteria as additives). The optimum treatment against Listeria is pediocin-producing Pediococcus acidilactici (2.8 mg/ml) which could be applied alone or in combination with bacteriophage or other bacteriocin-producing lactic acid bacteria. The obtained results will be further proceed, to direct application in different types dry cured sausages.

Introduction

In many commercial ready-to-eat (RTE) foods, recontamination with pathogens during post processing leads to outbreaks of food borne diseases. Good handling during post processing has been reported to increase the safety of these products. However, some robust pathogens like L.monocytogenes, may still grow during storage. L.monocytogenes is particularly problematic for food industry, due to it is widespread in the environment, and because of its ability to grow in a wide range of temperatures. Although listeriosis is rare, it is of public health concern, because of its high case fatality (20-30%). In addition, the number of notifications of L.monocytogenes in RTE products was reported to have increased (RASFF, 2006, 2007).

In fermented sausages, a sequence of barriers that appear along the ripening process (e.g. reduction of aw and pH) is applied. In typically Mediterranean sausages, the barriers applied during their production are quite low (e.g.moderate pH decrease). Hence, slightly resistant pathogens such as L.monocytogenes may be able to survive. Hurdle technology, which is a mild preservation technology based on the combination of multiple antimicrobial factors or processes, is considered to be effective to be reduce Listeria in production of dry cured sausages. The principle behind this technology is that when microorganisms are confronted with multiple antimicrobial factors, the probability for survival decreases due to an increase in the energy costs that leads to cell exhaustion and death. The synergy between different factors in addition may permit a decrease in their dose.

Therefore, before applying various antimicrobials in production of Spanish fermented sausages, an in vitro experiment, aiming at studying the inhibition effect of several antimicrobials against Listeria was performed. The antimicrobials used in this study were bacteriophage, nisin, pediocin, organic acids (e.g. sorbic acid, lactic acid), lysozyme, and lactic acid bacteria. The effect of their inhibition against L. innocua and L.monocytogenes under 30°C and low temperature (10°C) were evaluated.

Material and Methods

A. Microorganisms and growth medium

The microorganisms used in this study are two strains of Listeria innocua (strain C910 which was isolated from meat product and strain CCC05 which was supplied by EBI Food Safety-provider of ListexTMP100) and one strain of Listeria

monocytogenes (isolated from meat product). The medium used to grow these strains are Brain Heart Infusion (BHI) media (Oxoid) adjusted to pH 6 with acetic acid (in order to mimic the conditions of sausages) and ALOA agar (Oxoid) which is used for plate count. The bacterial cultures were prepared by diluting -80°C stored stocks in BHI media, grown at 37°C for overnight. 1 ml of this overnight culture was then transferred into fresh BHI media, grown at 37°C for 4-6 hours.

B. In vitro experiment

The additive used in these experiments are bacteriophage (ListexTM P100, EBI Food Safety), nisin (Larbus SA), sorbate (potassium sorbate E-202, Larbus SA), lactate (Conservador Prolac, Larbus SA), lysozyme (Larbus SA), bacteriocin producing bacterial cultures (pediocin-producing Pediococcus acidolactici in Fargo 37 (Amarex), Lactobacillus plantarum in HoldbagTM Listeria 10 IP (Danisco); Staphylococcus xylosus, Lactobacillus lactis, Lactobacillus plantarum in HoldbagTM 261 (Danisco); Micrococcus varians, Staphylococcus carnosus in Fermitrat N (Larbus SA). The concentration of additives used in this study was 101-1010 CFU/ml for bacteriophage (Listex), 0-400 µg/ml for nisin, 0-5 mg/ml for pediocin (Fargo 37), 0-5 mg/ml for sorbate (E-202), 0-50 mg/ml for lactate (conservador Prolac), 0-0.215 mg/ml for lysozyme, 0-5 mg/ml for culture Holdbag 10, and 0-2.5 mg/ml for culture Holdbag 261. The amount of inoculum used in the study was 105 CFU/ml. The growth inhibition of Listeria was measured spectrophotometrically at 630 nm after incubating the 96 well plate at 37°C for certain period of time.

Results

The in-vitro experiment performed in this study could be divided into two parts, determination of the optimum concentration of each additive and the optimum combination of additives. The result of first part was summarized in Table 1. The results demonstrated that among the additives applied, two general trends exist. Bacteriophage and pediocin-producing Pediococcus acidolactici (P) showed bactericidal effect against Listeria, whereas sorbate, lactate, lysozyme, culture of Lactobacillus plantarum (L), and mixed culture of Staphylococcus xylosus, Lactobacillus lactis, Lactobacillus plantarum (SLL) showed bacteriostatic effect.

Table 1. Determination of optimum concentration of each additive against Listeria

Treatment	Recommended dosage[a]	Effective dosage[b]	Comments
Bacteriophage	10^8 CFU/ml	10^9 or 10^{10} CFU/ml	At 10^8 CFU/ml slightly growth was observed after 48 h of incubation.
Nisin	200 µg/ml	-	At all concentration
Lysozyme	0.215 – 0.035 mg/ml	0.032 – 0.215 mg/ml	At lower concentration (0.032 and 0.043 mg/ml), it worked as bacteriostatic whereas at higher concentration (0.053, 0.081, 0.108, 0.215 mg/ml) it served as bacteriocidal.
culture of *Lactobacillus plantarum* (L)	1 mg/ml	0.5 – 5 mg/ml	At concentration, 0.5, 0.6, 0.8, 1, 2, and 5 mg/ml the growth inhibition was observed.
mixed culture of *Staphylococcus xylosus, Lactobacillus lactis, Lactobacillus plantarum* (SLL)	0.5 mg/ml	0.1 – 2.5 mg/ml	At all concentration applied (0.1 – 2.5 mg/ml), growth inhibition was observed.
Micrococcus varians, Staphylococcus carnosus (MS)	1 mg/ml	-	Not determined

a is the dosage which is suggested by the supplier catalogue, b is the dosage obtained as result of experiment.

After obtaining the optimum concentration of each additives, the in-vitro experiment was proceed with determination of the effective combination. Two different temperature were applied in this experiment, 30°C (as control) and 10°C (which simulates the curing process of dry-cured meat products).
At 30°C, combination of P-bacteriophage, P-MS, P-MS-bacteriophage, and application of bacterial suspension of P and MS (previously grown overnight at 30°C in MRS broth) inhibits better Listeria than P alone. However, for combination of P-MS or P-MS-bacteriophage, lower Listeria growth was obtained when less amount (2.8 mg/ml) of P was applied.
At 10°C, in general similar Listeria inhibition was obtained, irrespective of combination applied. Although the number of remaining Listeria after 2 weeks of incubation was similar, MS inhibition profile against Listeria at lower temperature are quite different in comparison to others. The application of bacterial cell suspension of P and MS gave no difference effects with the one that with P (without pre-incubation). Therefore it might be not necessary to use the bacterial cell suspension, as it will affect the natural fermentation process of meat products. For both temperatures studied, similar results were obtained with L.monocytogenes.

Discussion

Among all additives examined in this study, bacteriophage and pediocin-producing Pediococcus acidolactici (P) are the ones that effectively kills Listeria. Similar results of successful reduction of Listeria using pediocin or pediocin-producing strain in RTE products have been reported (Cosansu S et al, 2010; Nieto-Lozano JC et al, 2010; Olaoye OA and Dodd CER, 2010). The widely known and characterized pediocin reported in literature were pediocin AcH or PA1. This pediocin, produced by Pediococcus acidilactici, is the strain that was used in this study.

Regarding the effective combination of additives against Listeria, combination of pediocin-producing Pediococcus acidolactici (P) with bacteriophage or with other bacteriocin-producing cultures resulted in better inhibition than P alone. This demonstrated that multiple barrier technology is an effective strategy against listeria. The use of more than one bacteriocin producing strain overcomes some of the problems of limiting the effectiveness of bacteriocins in food systems (Kouakou P et al, 2010). Pediocin can also be used with other chemical barrier such as lactate and diacetate (Grosulescu C, 2011).

Conclusion

Our in-vitro experiment showed that the optimum treatment to inhibit Listeria growth is by using pediocin-producing Pediococcus acidolactici, which could be applied alone or in combination with other additives. The combination of multiple treatments should however be optimized as different effect can be encountered (e.g. synergistic, additive, or antagonistic effects). Our result demonstrated that the pediocin-producing Pediococcus acidolactici can be used as alternative for protection against Listeria in Spanish dry cured meat products, especially for the purpose of exportation to demanding countries such as USA.

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