Food Control 27 (2012) 163–169

Contents lists available at SciVerse ScienceDirect

Food Control

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

Pre-PCR treatments as a key factor on the probability of detection of *Listeria monocytogenes* and *Salmonella* in ready-to-eat meat products by real-time PCR

Belen Martin, Margarita Garriga, Teresa Aymerich*

IRTA, Food Safety Program, Finca Camps i Armet, 17121 Monells, Spain

ARTICLE INFO

Article history:
Received 19 January 2012
Received in revised form
5 March 2012
Accepted 10 March 2012

Keywords:
Salmonella
Listeria monocytogenes
Probability of detection
Real-time PCR
Pre-PCR treatment
RTE-meat products

ABSTRACT

The aim of this study was to compare the performance of different pre-PCR treatments for the detection of *Listeria monocytogenes* and *Salmonella* spp. by real-time PCR in spiked ready-to-eat meat samples (cooked ham, dry-cured ham and fermented sausage). Three different pre-PCR treatments were assayed: (i) PrepMan® Ultra Sample Preparation Reagent, (ii) DNA purification using DNeasy Tissue Kit and QIAcube automated sample preparation system, and (iii) BAX® system. The analysis range was 2 to 2×10^6 CFU/ml. PCR was done in quintuplicate and the experiment was repeated five times. The 'probability of detection' was used to compare the efficiency of the pre-PCR treatments and to calculate the theoretical limit of detection of each treatment.

The best pre-PCR treatment was DNA purification using DNeasy Tissue kit and QIAcube; it showed the highest detection probability and the lowest limit of detection for each food type and pathogen assayed, followed by PrepMan and BAX treatments. Probability of detection showed differences among food matrices and between *L. monocytogenes* and *Salmonella* detection probability. Despite being an expensive method, DNeasy Tissue kit would be the recommended treatment to reduce the risk of false negative results and to improve detection of foodborne pathogens in RTE-meat products by real-time PCR.

1. Introduction

Ready-to-eat (RTE) meat products have become an important part of the modern diet. Consumers appreciate their many benefits, including convenience and variety. Nevertheless, the manufacturing of this kind of products enhances the risk of microbial contamination, mainly during peeling, slicing and repackaging (USDA/FSIS, 2006). This can pose a risk for public health since RTE products are ordinarily consumed without additional preparation. RTE products are subjected to monitoring and surveillance programmes by the corresponding controlling Agencies. During 2010, 21 FSIS (Food Safety and Inspection Service)-recalls were due to pathogenic bacteria contamination (USDA/FSIS, 2010), and 52% of the recalls were for RTE-meat products or other ready-to-eat products containing meat contaminated with *Listeria monocytogenes* or *Salmonella*.

Microbiological criteria implemented for *Salmonella* in RTE-meat products requires absence of the pathogen in 25 g of sample (CE2073/2005 (European Commission, 2005)). For *L. monocytogenes*, the USDA retains a "zero tolerance" policy (9 CFR Part 430 (USDA/FSIS, 2006)), while other countries allow up to 100 CFU/g in those RTE foods unable to support its growth or in those RTE products able to support its growth without exceeding 100 CFU/g throughout the shelf-life of the product (European Commission, 2005).

Conventional methods for the detection of *L. monocytogenes* and *Salmonella* are labour-intensive and time-consuming in many instances requiring from 5 to 7 days to be completed. These methods involve several steps to obtain the final result: pre-enrichment, selective enrichment, isolation in selective agar and serological and biochemical confirmation (International Organization for Standardization, 1996, 2002). Alternative rapid methods have been developed for the detection of *L. monocytogenes* and *Salmonella* in food in shorter time periods such as chromogenic/or alternative media, immunoassays and molecular based methods (Jasson, Jacxsens, Luning, Rajkovic, & Uyttendaele, 2010). Among them, PCR methods are particularly relevant for the detection of pathogenic bacteria in food, since they allow an accurate and unambiguous identification of target nucleic acid sequences (Klein, 2002). Nevertheless, these alternative methods also require one enrichment step to increase the number of the target microorganism to detectable levels and a pre-PCR treatment to eliminate PCR inhibitors present in the food matrix or in the enrichment media.

* Corresponding author. Tel.: +34 972 630 052; fax: +34 972 630 373.
E-mail address: teresa.aymerich@irta.cat (T. Aymerich).

0956-7135/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodcont.2012.03.010

The objective of the enrichment step is favouring target pathogen multiplication limiting food background microbiota growth by the selection of appropriate enrichment conditions (Andrews, 1985). Naturally contaminated food generally contains low numbers of pathogens (<100 CFU/g) often affected by several stresses caused by environmental factors or preservatives such as temperature, pH, aw, lactic acid, etc (Farber, Daley, Coates, Emmons, & McKellar, 1992; Koutsoumanis & Sofos, 2005; Theys et al., 2008; Tienungoon, Ratkowsky, McMeekin, & Ross, 2000). Recovering stressed pathogen cells from food matrix is of great importance in food safety (Jay, Loessner, & Golden, 2005), since subletally injured bacteria can resuscitate and achieve high counts during the shelf-life of the product in favourable conditions (Marcos, Aymerich, Monfort, & Garriga, 2008). ISO methods for detection of *L. monocytogenes* and *Salmonella* include a pre-enrichment step to resuscitate injured cells in low-selectivity media. This step is crucial, since injured cells become sensitive to selective agents included in the enrichment broths (Beumer, te Giffel, Anthonie, & Cox, 1996; Crawford, Beliveau, Peeler, Donnelly, & Bunning, 1989) and some of these cells could not grow resulting in a high risk of false negative samples (Dupont & Augustin, 2009).

A pre-PCR treatment following enrichment step is necessary to obtain PCR-compatible samples. The presence of inhibitory substances in the food matrix or in the enrichment broth could interfere with the PCR amplification influencing the assay's sensitivity of detection (Rådström, Knutsson, Wolffs, M., & Löfström, 2004). Optimized pre-PCR treatments allow accurate DNA amplification without underestimating the bacterial loads or give false negative results (Abu al-Soud & Rådström, 2001; Haag & Raman, 1994; Saiki et al., 1985) by either sorting out PCR inhibitors from target bacteria and/or by concentrating the pathogens to detectable concentrations (Lantz, Al-Soud, Knutsson, Hahn-Hagerdal, & Rådström, 2000).

The aim of this study was to compare the performance of different pre-PCR treatments for the detection of *L. monocytogenes* and *Salmonella* spp. by real-time PCR in spiked samples of RTE-meat products (cooked ham, cured ham and fermented sausage). The procedures were assessed according to the probability of detection, given a known number of bacteria in the homogenized sample with a suitable enrichment broth.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Salmonella ser. Typhimurium CECT443 (food poisoning isolate) and *L. monocytogenes* CTC 1011 (serotype 1/2c, meat isolate) were used in this study. The strains were grown overnight at 37 °C in tryptic soy broth (DB, NJ, USA) supplemented with 0.6% yeast extract (Oxoid, Basingstoke, Hampshire, UK), TSBYE. The bacterial cultures were stored into aliquots at −80 °C with 20% glycerol for further use in spiking experiments. One aliquot of each bacterial stock was quantified after freezing by plating out 0.1 ml from selected dilutions in tryptone soya yeast extract agar, TSAYE, and incubated at 37 °C for 24 h.

2.2. RTE-meat products samples

Three kind of commercial RTE-meat products were assayed: sliced cooked ham, vacuum-packed sliced dry-cured ham and low-acid fermented sausage. Products were purchased in local stores and transferred to our laboratory where they were kept at 4 °C until further use. Twenty five grams of each sample were homogenised with 225 ml of the appropriate enrichment broth in a Masticator Classic (IUL S.A., Barcelona, Spain). For comparison reasons, only one enrichment broth was used for the detection of each pathogen. Enrichment media selected were 24 Listeria enrichment broth (24 LEB, Oxoid) for *L. monocytogenes* detection (an optimized enrichment broth recommended by BAX® System *L. monocytogenes* 24E) and Buffered Peptone water (BPW, AES Laboratories, Combourg, France) for *Salmonella* detection (one of the most common primary enrichment broths in *Salmonella* detection methods, such as BAX *Salmonella* Kit and ISO 6579).

2.3. Spiking of homogenized samples with Salmonella and L. monocytogenes

The homogenized samples were artificially inoculated with decreasing amounts of the corresponding stock cultures of *Salmonella* or *L. monocytogenes* to obtain a CFU range equivalent to approximately 2×10^6, 2×10^5, 2×10^4, 2×10^3, 2×10^2, 2×10^1 and 2 CFU/ml. A non-spiked sample was also included in each experiment as a negative control. The samples were directly submitted to the different pre-PCR treatments evaluated. To avoid potential sampling errors, the spiking trial was repeated five independent times for each RTE product and bacteria.

2.4. Pre-PCR treatments

Three DNA extraction methods were used: the PrepMan® Ultra Sample Preparation Reagent (Applied Biosystems, Foster City, CA), the DNeasy Blood and Tissue Kit (Qiagen GmbH, Hilden, Germany) and the BAX® system lysis method (Dupont Qualicon™, Wilmington, DE, USA).

2.4.1. PrepMan pre-PCR treatment

The PrepMan® Ultra procedure is a heat-based extraction method. For this method, 2-ml, 4-ml and 10-ml aliquots of sample homogenates were centrifuged at 9000× *g* for 5 min; the pellets were resuspended in 200 μL of the PrepMan® Ultra Sample Preparation Reagent and heated at 100 °C for 10 min. After cooling, the samples were centrifuged at 13,000× *g* for 5 min to eliminate cell debris and 50 μL of the supernatant were collected and diluted with 400 μL of TE (10 mM Tris HCl and 1 mM EDTA, pH 8.0). DNA was precipitated using 50 μL of 3 M sodium acetate and 500 μL of isopropanol for 15 min at room temperature and centrifuged at 13,000× *g* for 10 min. The resulting pellets were air dried and the DNA was resuspended in 50 μL of UltraPure™ DNase/RNase-free distilled water (Invitrogen, Merelbeke, Belgium). A 5-μL aliquot was used as template for real-time PCR analysis.

2.4.2. QIAcube pre-PCR treatment

The DNeasy Blood and Tissue Kit is a column-based DNA extraction method. Qiagen spin column can be processed using QIAcube automated sample preparation system, a Robotic workstation for automated purification of DNA, RNA and proteins. For this method, the sample pellets were prepared as in 2.4.1., using also 2-ml, 4-ml and 10-ml aliquots of sample homogenates. For *Salmonella* detection, tubes containing the bacterial pellets were directly placed on the QIAcube shaker. For *L. monocytogenes*, pellets were previously resuspended in180 μL of enzyme solution (35 mg/ml lysozyme in 20 mM Tris−HCl pH 8.0, 2 mM EDTA, 1.2% Triton) and the QIAcube enzymatic lysis step was programmed at 37 °C for 1 h. DNA was eluted in 150 μL of buffer AE (10 mM Tris HCl, 0.5 mM EDTA pH 9.0) and 5 μL of this solution was used for real-time PCR analysis.

2.4.3. BAX pre-PCR treatment

In BAX® system pre-PCR treatment, samples are heated in a lysis reagent solution following manufacturer's instructions. For