The development of a combined surface adhesion and polymerase chain reaction technique in the rapid detection of *Listeria monocytogenes* in meat and poultry

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Abstract

A procedure combining enrichment surface adhesion and polymerase chain detection (SA-PCR) was developed and applied in the detection of *Listeria monocytogenes* in meat products. Minced beef samples were inoculated with *L. monocytogenes* ($\log_{10} 3$ cfu g^{-1}) and incubated for 10 h at 30°C in buffered peptone water. *L. monocytogenes* was recovered from the culture by attachment to a polycarbonate membrane immersed for 15 min in the enriched meat culture. The membrane and attached bacteria were removed from the culture and the membrane dissolved in phenol:chloroform. The DNA was extracted from the bacteria and a PCR assay was carried out using primers directed against the listerolysin O gene of *L. monocytogenes*. The combined (SA-PCR) technique had a detection limit of $\log_{10} 4.0$ cfu ml^{-1} in enriched meat cultures. The rapid technique was applied to a small number of retail samples ($n = 100$) and was found to compare favourably to the standard cultural method. A total of 12 samples were confirmed positive for *L. monocytogenes* using the standard cultural method and the SA-PCR assay. No false positives or negatives were recorded by either method. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Listeria monocytogenes is a pathogen which has been linked to outbreaks of food poisoning involving a wide range of foods including coleslaw, milk, cheese, chicken and pate (Schlech et al., 1983;

Fleming et al., 1985; Brackett, 1988; Linnan et al., 1988; Kaczmarski and Jones, 1989, McLauchlin et al., 1991). It has also been detected on a range of food products including raw and ready to eat products at retail level (Gilbert et al., 1989; Carosella, 1990; Sheridan et al., 1994), at concentrations of 10–1000 cfu g^{-1} (Sheridan et al., 1994; Jay, 1996; Walsh et al., 1998). The minimum infective dose has not been reliably established, however, published information on the numbers of *L. monocytogenes* in

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contaminated foods during outbreaks indicate that levels of between 10^3–10^4 were responsible for illness (Lorber, 1990; Rocourt and Cossart, 1997). Therefore, the above reported concentrations of *L.monocytogenes* may constitute a significant public health risk in ready-to-eat foods. Alternatively, such contaminated foods may pose a broader risk as sources of cross contamination within food production and service operations (McLauchlin, 1996).

Currently available cultural methods for the detection of *L. monocytogenes* are labour intensive and generally slow in achieving an end result, requiring four days to establish presumptive presence, and a further 2–3 days to confirm and speciate isolates (Farber and Peterkin, 1991). Effort in the development of alternative rapid methods for the detection of this pathogen have largely been directed towards the use of antibody and nucleic acid based tests (Samarajeewa et al., 1991; Hill, 1996; Scheu et al., 1998). However, these techniques generally require lengthy sample enrichment periods (48 h) to increase the numbers of target cells to detectable levels (10^5–10^6 cfu ml^{-1}) (Dever et al., 1993). Thus, although the final detection process is relatively rapid, the extended enrichment necessary means that, overall, these "rapid" methods can take up to three days to yield results.

Inherent problems associated with such techniques include difficulties in the recovery of species such as *L. monocytogenes* from the complex food matrix produced by initial incubation of food products with selective enrichment broths. A range of methods have been applied in the isolation of the pathogens including immunomagnetic separation, filtration, flocculation and impedance methods (Hale et al., 1990, Walls et al., 1990, Skjerve et al., 1990, Silley and Forsythe, 1996). While these recovery methods have had some success, they are time consuming to carry out and can significantly extend the duration of the isolation and detection procedure. Inefficiencies in extraction of the target pathogen from the food matrix, and poor separation from elements of the competitive microflora (Beumer and Brinkman, 1989) can lead to subsequent problems in the accurate detection and/or differentiation of target organisms. Thus co-extracted materials can interfere with DNA hybridisation tests in PCR assays (Rossen et al., 1992; Blais and Phillippe, 1993) and effective immunoassay (Beumer and Brinkman, 1989).

Many of these pathogen extraction difficulties can be significantly reduced by the use of a surface adhesion based extraction procedure (Sheridan et al., 1997, Sheridan et al., 1998). This procedure involves the brief immersion (15 min) of a polycarbonate membrane (mounted on a glass carrier slide) in an enriched food sample. During this process, bacteria in the enriched sample rapidly attach to the polycarbonate membrane surface. This procedure is a simple and rapid method to extract bacteria from the enriched sample.

Previously, bacteria recovered on polycarbonate membranes have been detected and/or enumerated by immunofluorescent methods i.e., the surface adhesion immunofluorescence (SAIF) technique (Sheridan et al., 1997). Although this approach is effective, it is heavily reliant on the specificity of the monoclonal antibody employed. Thus for example, available monoclonal antibodies can be too specific, or too general for some purposes. In addition, the sourcing and preparation of the necessary range of immunofluorescent antisera to interact with a wide and changing range of pathogenic species and strains can prove expensive and time consuming. An approach which can link the rapid initial recovery and concentration potential of the surface adhesion (SA) procedure, with the discriminative potential of polymerase chain reaction (PCR) assay may yield a highly sensitive and specific method for detection of the pathogen.

This paper describes the use of the surface adhesion technique for the extraction of *L. monocytogenes* from an enriched food sample onto a polycarbonate membrane, combined with subsequent detection of the pathogen by a PCR technique. The application of the technique to broth culture, inoculated meat samples and a small number of naturally contaminated retail samples are discussed. The advantages and aspects for the development of the technique are described.

2. Materials and methods

2.1. Bacterial cultures

Bacterial strains used in this study included *L. monocytogenes* 4b (NCTC 11994), *L. monocytogenes* 4b (raw milk isolate), *L. monocytogenes* Scott

A (meat isolate) *Listeria innocua* (NCTC 3670), *L. ivanovii* (NCTC 4073), *Listeria seeligeri* (NCTC 2837), *Listeria welshimeri* (NCTC 2816), *Listeria grayi* (NCTC 10815), *Listeria murrayi* (NCTC 10912), *Brochothrix thermosphacta* (ATCC 11509), *Escherichia coli* (NCTC 09001), *Hafnia alvei* (NCTC 08105), *Lactobacillus spp* (meat isolate), *Salmonella enteritidis* (NCTC 5765) and *Pseudomonas fragi* (NCIMB 8542). All strains were maintained on Protect beads (Protect Bacterial Preserves, UK) and stored at $-20°C$. All strains were re-streaked at regular intervals to ensure viability.

2.2. *L. monocytogenes* broth studies

One *L. monocytogenes* (NCTC 11994) coated Protect bead was placed in 10 ml of Brain Heart Infusion broth (Oxoid, CM225, Basingstoke, UK) and incubated overnight at 30°C. A 1.0 ml aliquot of this culture was resuspended in 9 ml of 0.1% peptone water (Oxoid, CM9, Basingstoke, UK) and the numbers of cells per ml determined by a rapid membrane filtration acridine orange count (Walls et al., 1989). Based on this data, the appropriate amount of overnight culture was added to 225 ml volumes of buffered peptone water (BPW, Oxoid, CM509, Basingstoke, UK) to give a series of broth cultures with initial cell concentrations ranging from $\log_{10} 3.0$ cfu ml^{-1} to $\log_{10} 8.0$ cfu ml^{-1}. The numbers of *Listeria monocytogenes* in these cultures were determined by:

2.2.1. A standard plate count (SPC) method

A 1.0 ml aliquot of the culture was removed and serially diluted in 0.1% peptone water and plated in duplicate onto Palcam agar (Oxoid,CM877 with supplement SR150E, Basingstoke, UK) using the spread plate technique. Plates were incubated at 30°C for 48 h and typical *Listeria* colonies enumerated. Colonies were subsequently confirmed as *Listeria* using a series of biochemical tests including the Gram reaction, catalase reaction, motility at 25°C, nitrate reduction, Vogues-Proskauer reaction, haemolysis of horse blood agar, the CAMP test and fermentation of carbohydrates (mannitol, rhamnose, xylose and α-methyl-D-mannoside) (McClain and Lee, 1987).

2.2.2. A surface adhesion (SA) method

L monocytogenes was isolated from broth cultures using the surface adhesion method previously described by Sheridan et al. (1997). This involved the attachment of a polycarbonate membrane (25mm in diameter, 0.6 μm pore size, Poretic products, 11 Lindberg Ave, Livermore, CA, USA) to a glass slide using 1% molten bacteriological agar (Oxoid, L11, Basingstoke, UK). Duplicate membranes were then completely immersed in the test culture for 15 min to allow bacteria in the culture to attach to the membrane.

3. Recovery/detection

3.1. Rinse counts

One of each set of duplicate membranes was placed in 10 ml of 0.1% peptone water and shaken vigorously by hand for 2 min. This allowed the *L. monocytogenes* cells to detach from the membrane into suspension. Aliquots of 0.1ml from the suspension were plated onto Palcam agar using a spread plate technique. Plates were incubated at 30°C for 48 h and examined for the presence of typical *L. monocytogenes* colonies (Sheridan et al., 1997). Typical colonies were confirmed as *Listeria* using a series of biochemical tests as mentioned previously.

3.2. Polymerase chain reaction assay

3.2.1. DNA extraction

Bacteria adhering to the second membrane of each duplicate set were examined by PCR assay using a modification of the procedure of Hoffman and Winston (1987). Briefly, the membrane was placed into a 1.5 ml sterile Eppendorf tube (Sarstedt, UK) containing 200 μl of lysis buffer (2%(v/v) Triton X-100, 1% SDS, 100 mM NaCl, 10 mM Tris pH 8.0, 1 mM EDTA). A portion (200 μl) of phenol:chloroform: isoamylalcohol (Sigma, UK) in a ratio of 25:24:1 was added to the Eppendorf tube and the mixture vortexed for 30 s. Acid washed glass beads (0.3 g, 425–600 microns, Sigma, U.K.) were added to the mixture, which was vortexed for a further 2 min. This procedure released all genomic DNA and also dissolved the polycarbonate membrane.

3.2.2. DNA recovery

Each sample mixture was centrifuged at 2700 *g* for 5 min (Eppendorf, model 5417, Germany). A 200 μl aliquot of the supernatant was removed and placed in a 0.5 ml sterile Eppendorf tube (Geneamp tubes, Perkin Elmer, USA) with 3 M sodium acetate (20 μl) (Sigma, UK) and 600 μl of absolute alcohol (Sigma, UK). The mixture was vortexed for 1 min and centrifuged at 2700 *g* for 10 min. The supernatant was removed and the pellet resuspended in 500 μl of 70% alcohol (Sigma, UK). The mixture was vortexed for 2 min and centrifuged for a further 2 min at 2700 *g*. The supernatant was decanted and the Eppendorf centrifuged for a further 2 min, and any remaining supernatant alcohol removed by aspiration. Residual alcohol was removed by placing the open Eppendorf tubes in a DNA dryer (DNA Vac dryer, Savant, USA) at 40°C, for 10 min. The dried precipitates were resuspended in 50 μl of sterile distilled water and held at room temperature for 20 min.

3.2.3. Primers

Synthetic oligonucleotide primers used in this study were the primers of Thomas et al. (1991), i.e. one forward (LL5) and one reverse (LL4) primer, complimentary to the published listeriolysin O gene sequence (Mengaud et al., 1988). Primers were synthesised on a 30 μM scale using a Beckman synthesiser at The National Food Biotechnology Centre, University College Cork, Ireland.

3.2.4. DNA amplification by PCR

DNA amplification was performed in a total volume of 47.70 μl containing the following reagents: 5 μl $MgCl_2$ (5mM), 5 μl of 10X Taq buffer (160 mM $(NH_4)_2SO_4$, 670 mM Tris–HCl (pH 8.8 at 25°C), 0.1% Tween-20), 1 μl dNTPs (0.25 mM), 2.5 μl of each primer (1 μM), 0.250 μl Taq polymerase (1 unit) (Bioline, UK) and 2 μl of template DNA. The amplification mixture was subsequently vortexed for 30 seconds and placed in a thermal cycler (PTC-200, MJ research, DNA Engine, USA). Template DNA was initially denatured at 95°C for 5 min and 35 PCR amplification cycles were performed as follows: denaturation at 94°C for 1 min, primer annealing at 60°C for 1 min and DNA extension at 72°C for 2 min.

3.2.5. Detection of PCR products

PCR products were detected by electrophoresis of 20 μl of the amplification mixture on a 0.7% agarose gel (Sigma, U.K.) run in a Tris acetate buffer (Sigma) containing 200 ng of ethidium bromide (Gibco-BRL, Life Technologies, Scotland) per ml of buffer. Gels were visualised under U.V. light and catalogued with a gel documentation system (Stratagene EE 2, Germany). A 100 bp DNA ladder (Pharmacia Biotech, USA) molecular weight marker was included in each electrophoretic gel to allow identification of the amplified product. A positive result, i.e., the presence of *L. monocytogenes* in the original broth culture, was indicated by a characteristic PCR product (520 bp).

4. Cross reactivity

A series of overnight cultures of a range of bacteria, including common meat pathogens and meat spoilage organisms (*E. coli*, *B. thermosphacta*, *Lactobacillus spp*, *S. enteritidis*, *Ps. fragi*, *H. alvei*, *L. ivanovii*, *L. seeligeri*, *L. welshimeri*, *L. grayi*, *L. murrayi* and *L. innocua*) were prepared by placing Protect coated beads of each species into 30 ml volumes of BHI (Oxoid, Basingstoke, UK) and incubating cultures at their optimum temperatures. Preliminary studies (not reported) established that overnight incubation resulted in final bacterial concentrations of approximately $\log_{10}7$ cfu ml^{-1}. Recovery of bacteria by surface adhesion from each overnight culture, and DNA extraction/PCR amplification were carried out as described above. DNA/ PCR amplification procedures were also performed on control, i.e., unimmersed polycarbonate membranes. The resultant amplification mixtures were examined to detect any possible interactions between the primers employed and other bacterial species or interference by components of the polycarbonate membranes.

4.1. Inoculated meat studies

Beef diaphragm meat (650 g) obtained from the abattoir at The National Food Centre was prepared by excision of diaphragm muscle, aseptic removal of visible fat, slicing, and mincing, through a sterile steel plate (5 mm, Crypto Peerless Mincer, model

EB 12F, London). Samples of minced beef (50 g) were aseptically inoculated with 1.0 ml of an *L. monocytogenes* culture (prepared as described above) to a level of $\log_{10}$ 4 cfu g^{-1}. The inoculated meat was stomached for 2 min (Colworth 400, UK) in 500 ml of BPW to form a meat culture containing a final concentration of $\log_{10}$ 3.0 cfu ml^{-1}. Previous work carried out by the authors has suggested that selective broths offered no significant advantages over a non selective broth such as BPW for the recovery of *L. monocytogenes* from meat samples and as part of a rapid method protocol (Duffy et al., 1994; Sheridan et al., 1997; Walsh et al., 1998). The meat culture was incubated in a water bath (MT-Lauda, AGB, Ireland) at 30°C (± 0.2°C) and samples (70 ml) were withdrawn at intervals of 0, 2, 4, 6, 8 and 10 h. A portion (1 ml) of each sample was enumerated by plate count as described above and the rest of the sample (70 ml) was poured into a sterile staining trough. Duplicate polycarbonate membranes were immersed in the meat cultures for 15 min, removed, and examined for the presence of *Listeria* using the rinse counts and PCR amplification procedures described above.

5. Application of SA-PCR to retail food samples

Portions of fresh chicken ($n = 80$) and minced beef ($n = 20$) were purchased from various retail outlets in the Dublin area. Two 25 g samples were aseptically taken from the food to be tested.

One of each set of duplicate 25g samples was examined for the presence of *L. monocytogenes* by a method used by Sheridan et al. (1994). In this procedure the food sample was homogenised in 225 ml of *Listeria* primary enrichment broth (UVM I formulation), (Oxoid, CM863 with supplement SR142E, Basingstoke, UK) and incubated for 24 h at 30°C. After incubation, a 0.1 ml aliquot was transferred to 9.9 ml of *Listeria* secondary enrichment broth (UVM II formulation) (Oxoid, CM863 with supplement SR143E, Basingstoke, UK) and incubated at 30°C for a further 24 hours. Following enrichment, each sample was streaked onto Palcam agar and incubated for 48 h at 30°C. Presumptive *Listeria* colonies (black dimpled colonies) were restreaked onto TSA (Oxoid, CM131, Basingstoke, UK) and incubated for 24 h at 30°C. A number of

biochemical tests were carried out to confirm and speciate the presumptive *Listeria* colonies (McClain and Lee, 1987).

The other 25 g sample in each case was homogenised for 2 min in BPW (225 ml) (Seward, UK) and incubated at 30°C. Duplicate polycarbonate membranes were immersed in the food cultures after 18 and 24 h enrichment, removed, and examined for the presence of *L. monocytogenes* using the previously described PCR amplification technique.

6. Results

Investigation of the specificity of listerolysin O primer against a range of *Listeria* spp. in broth cultures established that all the *L. monocytogenes* serotypes examined yielded a 520 bp product. None of the other *Listeria* spp. investigated, including *L. innocua*, *L. ivanovii*, *L. seeligeri*, *L. welshimeri*, *L. grayi* and *L. murrayi* produced a detectable PCR product at the 520 bp region. Of the other bacterial species tested, only the *Lactobacillus* spp. yielded PCR products i.e., two non-specific bands of 850 and 940 bp. Polycarbonate membranes dissolved during the DNA extraction process were not found to interfere in the PCR reaction or to produce any detectable products during electrophoresis.

Studies carried out in inoculated broths showed that the surface adhesion PCR technique was able to produce a discernible 520 bp band on electrophoresis of PCR product from broths containing approximately $\log_{10}$ 4.0 cfu ml^{-1} (Fig. 1). Samples from broth cultures containing *L. monocytogenes* at higher cell concentrations produced more intense bands.

Fig. 2 shows the results of SA-PCR assay of inoculated meat cultures. No PCR products were visible in samples that were incubated for 2 h. In samples which had been incubated for 4 h, SA-PCR showed a weak band of PCR product at the 520 bp position. Plate counts from these samples indicated levels of approximately $\log_{10}$ 4.02 cfu ml^{-1} in these meat culture samples (Table 1). Samples which had been incubated for 6 h and contained levels of $\log_{10}$ 5.32 cfu ml^{-1} displayed visibly more intense PCR product bands at the 520 bp position. These results indicate that a minimum level of $\log_{10}$4.0 cfu ml^{-1}*L. monocytogenes* in meat culture was necessary to achieve a positive result with the SA-PCR technique.
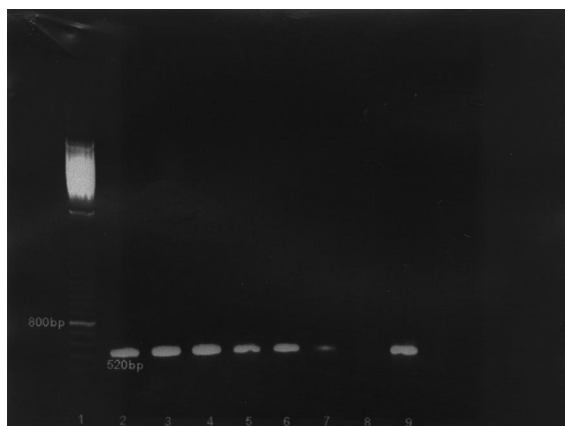
Fig. 1. Relationship between number of *L. monocytogenes* per ml of enrichment broth and PCR product band as determined by a rapid surface adhesion PCR technique. Lane 1: 100bp molecular marker; Lanes 2–7: Membrane dipped in broth culture containing $\log_{10}8$ cfu g^{-1}–$\log_{10}3$ cfu g^{-1} of *L.monocytogenes*; Lane 8: *L.innocua* (pure culture, negative control); Lane 9: *L.monocytogenes* (pure culture, positive control).
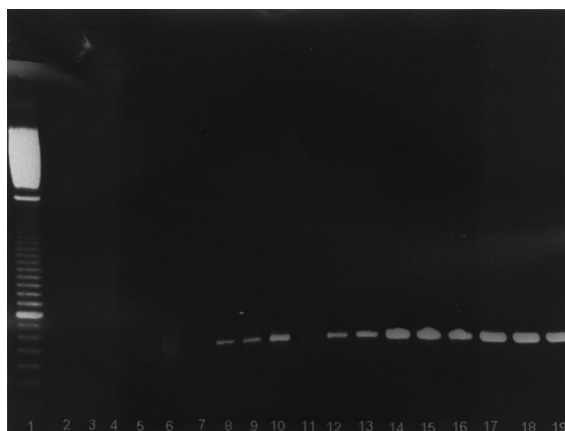

Fig. 2. Relationship between enrichment time and PCR product band obtained for *L. monocytogenes* using a surface adhesion PCR technique in inoculated meat culture. Lane 1: 100 bp molecular weight marker; Lanes 2–4: Samples taken after 0 h; Lanes 5–7: samples taken after 2 h; Lanes 8–10: samples taken after 4 h; Lanes 12–13: samples taken after 6 h; Lanes 14 –16: samples taken after 8 h; Lanes 17–18: samples taken after 10 h. Intial inoculum at time 0 was 10 cfu ml^{-1}

Table 1
Relationship between enrichment time (meat culture samples), number of *L. monocytogenes* in culture and numbers isolated onto the membrane surface

Time	Plate counts[a]		Membrane rinse counts	
(h)	($\log_{10}$ cfu ml^{-1})	S.D.[b]	($\log_{10}$ cfu mm^2)	S.D.
0	2.96	0.37	0.67	0.21
2	3.83	0.23	1.58	0.31
4	4.02	0.25	2.35	0.30
6	5.32	0.52	3.22	0.39
8	5.91	0.29	3.79	0.35
10	7.31	0.42	6.04	0.43

[a] Mean of 13 replicates.
[b] S.D. standard deviation

Table 2
Listeria spp.detected in 100 retail food samples using a standard enrichment and plating method, and a SA-PCR method[a]

Listeria spp	Standard method		SA-PCR method	
	Beef	Chicken	Beef	Chicken
L. monocytogenes	2	10	2	10
L. innocua	0	13	0	0
L. welshimeri	0	7	0	0
L. murrayi	0	4	0	0
L. grayi	0	2	0	0
Total positive	2	36	2	10

[a] Beef $n = 20$, Chicken $n = 80$.

and plating technique detected *Listeria* spp in 38 of the 100 samples examined (36/80 chicken samples and 2/20 minced beef samples). However, the majority of these (26/38) were found to be species other than *L. monocytogenes*, i.e., *L. innocua* (13) and to a lesser extent *L. welshimeri* (7), *L. murrayi* (4) and *L. grayi* (2). Twelve samples (two minced beef/ten chicken) were found to contain *L. mono-cytogenes* by the standard enrichment/plating tech-nique (which takes 96 h). *L. monocytogenes* was detected in all 12 of these samples by SA –PCR (within 29 h). *L. monocytogenes* was the only *Listeria* species detected by the SA-PCR method (Table 2), indicating the highly specific nature of the primers employed in this study.

7. Discussion

Surface adhesion using a polycarbonate membrane was successfully employed in this study to overcome some of the problems associated with the application

Membrane counts as determined by the rinse count method are also shown in Table 1.

Table 2 presents the numbers of retail food samples found to contain *Listeria* spp food samples using the standard enrichment/plating method and the SA-PCR method. In all, the standard enrichment

of the PCR assay to food analysis. Immersion of a membrane in the test culture provided a rapid and simple way of isolating *L. monocytogenes*. Recovery of *L. monocytogenes* by surface adhesion onto the membrane compared favorably to the standard cultural method. With increased enrichment time the numbers of *L. monocytogenes* in the culture increased as did the numbers detected on the membrane. The numbers of *L. monocytogenes* adhering to the membrane in this study were similar to those recorded by other workers who have previously employed this surface adhesion technique successfully in combination with immunofluorescent microscopy (Sheridan et al., 1997, 1998).

The combined surface adhesion PCR technique offers several advantages over other extraction methods such as immunomagnetic separation (IMS) where the non specific binding of meat microflora has presented problems (Fratimico et al., 1992; Parmer et al., 1992; Coleman et al., 1995; Duffy et al., 1997). Foods with a high fat content have also caused problems in IMS techniques as the magnetic beads can often become engulfed in fatty substances preventing successful recovery of the beads and detection of the target organism (Skjerve et al., 1990; Olsvik et al., 1994). Direct detection of *L. monocytogenes* using PCR from foods such as meat is often limited because of the presence of naturally occurring PCR reaction inhibitors, nucleic acids from other bacteria as well as from the food itself and the practicality of extracting small numbers of cells or minute amounts of DNA from the food mass (Rossen et al., 1992; Cooray et al., 1994). Application of the surface adhesion methods avoids these classic problems, by concentration of the bacteria in the enriched food sample onto a membrane, eliminating some of the components of food which may inhibit the process.

The DNA extraction procedure used led to the complete solubilisation of the membrane allowing the release of the bacterial DNA. In general, the use of membranes in the PCR process has been documented by other workers (Bej and Mahbubani, 1992; Oyofo and Rollins, 1993) some of whom reported that inclusion of the membrane in the assay inhibits the process as DNA binds to the membrane filter. Such binding was not evident in this study, and is unlikely to be of significance, as the chemical extraction method leads to membrane dissolution. The use of such a chemical extraction method

resulted in DNA of sufficient quality and quantity for accurate PCR analysis. Other workers have employed this technique with similar success (Deneer and Boychuk, 1991; Starbuck et al., 1992). A possible disadvantage of this chemical solubilisation method is the feasibility of its use in commercial environments, where the use of chemicals such as phenol could prove difficult.

The primers chosen for the assay proved to be highly specific for *L. monocytogenes*, giving a single amplified product of the expected size and exhibiting no cross reactivity when tested against a number of other common meat bacteria. This degree of specificity for *L. monocytogenes* is similar to the high levels of specificity observed in other studies with these primers (Deneer and Boychuk, 1991; Thomas et al., 1991, Lawrence and Gilmour, 1994).

The minimum detection level for *L. monocytogenes* from broth cultures by the SA-PCR ($\log_{10}3$–$\log_{10}4$ cfu ml^{-1}) compares favourably with the detection limit of other assays in broth culture such as the surface adhesion immunofluorescent technique ($\log_{10}$ 3.11 cfu ml^{-1}) (Sheridan et al., 1997). For *L. monocytogenes* in inoculated meat studies the minimum detection level by SA-PCR ($\log_{10}4$ cfu ml^{-1}) is similar to that reported by Fitter et al. (1992) who required a minimum level of $\log_{10}4$ cfu g^{-1}. Fitter's method was based on a a brief enrichment procedure followed by PCR analysis for the detection of *L. monocytogenes* in 24–48 h for a range of foods. However, some other workers e.g., Werners et al. (1991) have reported significant variations in sensitivity in the detection of *L. monocytogenes* in some foods. In some cases, it was possible to directly detect *L. monocytogenes* inoculated into some soft cheeses at levels as low as $\log_{10}3.3$ cfu/g. However, with other soft cheeses it was not possible to detect much higher concentrations of *L. monocytogenes* i.e., $\log_{10}$ 8 cfu/g. The reasons for variation in PCR sensitivities are unclear but it may possibly be due to the food in question or the type of primer employed in the assay and the gene that it targets.

This study has established that the SA-PCR technique can be successfully applied in the examination of retail meat samples. A minimum level of $\log_{10}$ 4 cfu g^{-1} in the enriched retail samples was required for the detection of *L. monocytogenes* using the SA-PCR technique. A short enrichment period (18 and 24 h) was required to allow the very low initial numbers of *L. monocytogenes* in the retail samples

(Duffy et al., 1994) to increase to levels which could be detected using the SA-PCR technique. Detection of amplified product was the same for the different foods tested, i.e., a PCR product was found at the 520 bp region for the meat samples which tested positive for *L. monocytogenes*, indicating the food type in this study had no effect on the assay. Other workers have reported that the food being tested often effects the accuracy of PCR amplification (Werners et al., 1991). The level of detection using the SA-PCR assay is significantly greater than reported by Bessesen et al. (1990) and Thomas et al. (1991)who required minimum levels of $\log_{10}$ 6 and $\log_{10}$5.4, respectively, to achieve a distinct amplicon. The assay results compared favourably to those achieved by Fitter et al. (1992) who also found that a minimum contamination level of $\log_{10}4$ cfu g^{-1} in foods was required for detection by their method.

One problem commonly associated with the application of PCR directly to foods is the sample size. Only small volumes, 5–10 μl/0.1g of the culture/food sample can be analysed per amplification reaction (Candrian, 1995). Limitations in sample size coupled with non-uniform distribution of the bacteria in the food matrix could result in false negative results being obtained (Thomas et al., 1991; Olsen et al., 1995). The surface adhesion method for the concentration and extraction of bacteria in a food sample (25 g) onto a polycarbonate membrane, allows a representative portion of the food to be analyzed. Membrane extraction reduces the potential inhibition of PCR by food components and also offers a method of concentrating the bacteria after a brief enrichment procedure, therefore maximizing the capture of *L. monocytogenes* from foods.

In conclusion, the SA-PCR assay is a rapid, sensitive and specific method for the detection of *L. monocytogenes* in meat samples It takes 29 h to complete and is a promising alternative to the currently employed cultural methods.

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