ELSEVIER

International Journal of Food Microbiology 56 (2000) 167–177

INTERNATIONAL JOURNAL OF
Food Microbiology

www.elsevier.nl/locate/ijfoodmicro

Combined PCR and slot blot assay for detection of *Salmonella* and *Listeria monocytogenes*

Xiaoming Li, Nissab Boudjellab, Xin Zhao*

Department of Animal Science, McGill University, Ste Anne de Bellevue, Quebec, Canada

Received 14 January 1999; received in revised form 29 July 1999; accepted 10 December 1999

Abstract

Detection of *Salmonella typhimurium* and *Listeria monocytogenes* by the polymerase chain reaction (PCR) assay coupled with slot blot detection was investigated in this study. After being extracted from diluted bacterial culture with the extraction buffer, bacterial DNA was subjected to PCR. The slot blot assay was optimized and used to detect PCR products. The lowest detection level of this method was 10^3 cfu/ml in the original culture media for both pathogens, or 5 bacterial cells in the PCR reaction. Combined with immunomagnetic separation (IMS) to separate and concentrate bacteria from samples, the detection limit could be 40 cfu/ml of bacteria from milk samples. The whole detection procedure was completed within 7 h. After multiplex PCR (amplification of DNA from two different bacteria in the same PCR tube) and slot blot, a detection level of 10^3 cfu/ml was achieved in the simultaneous detection for both pathogens, which was similar to that of individual detection for each pathogen. The combination of PCR and slot-blot seems to be highly sensitive and time-efficient, and is therefore promising for routine use in the detection of *Salmonella* and *L. monocytogenes* in food samples such as milk.

Keywords: *Salmonella*; *Listeria monocytogenes*; PCR; Slot blot; IMS

1. Introduction

Salmonella has long been recognized as an important foodborne human pathogen of worldwide economic significance. The most common diseases caused by *Salmonella* in human are enteritis, septicemia and abortion (Clarke and Gyles, 1993). In the United States, *Salmonella* causes an estimated 800,000 to 4 million infections, and kills about 500 people annually (Helmick et al., 1994). Moreover, the emergence of multidrug-resistant *Salmonella enterica* serotype *typhimurium* makes the outbreak of this human pathogen more dangerous (Glynn et al., 1998). *Listeria monocytogenes* is another foodborne pathogen that causes listeriosis as well as gastrointestinal illness. Recently, a number of outbreaks with *L. monocytogenes*-induced gastroenteritis have been reported (Riedo et al., 1994; Dalton et al., 1997). The ability of *L. monocytogenes* to grow over a wide range of temperatures makes it

*Correponding author. Tel.: +1-514-398-7975; fax: +1-514-398-7964.

E-mail address: Zhao@agradm.lan.mcgill.ca (X. Zhao)

0168-1605/00/$ – see front matter
PII: S0168-1605(99)00209-3

potentially hazardous especially for refrigerated foods (Czuprynski, 1993).

In order to minimize the hazards caused by both *Salmonella* and *L. monocytogenes,* the development of a rapid, sensitive and specific method for early detection of these bacteria is of the utmost importance. The traditional culture methods involve isolating the organisms of interest in pure culture and performing predetermined biochemical and serological tests to identify them (Catteau, 1995; Gledel and Corbion, 1995). These methods generally take 4–6 days to obtain the definitive results, and are time consuming and laborious. Recently, a number of new methods for rapid detection of bacteria have been reported, including immunological methods, nucleic acid-based hybridization methods and polymerase chain reaction (PCR) methods (Fung, 1995). For immunological methods and nucleic acid-based hybridization methods, enrichment of pathogens in samples is usually required because of the low sensitivity of these methods, which therefore lengthens the detection time. By PCR, one DNA molecule can be amplified many million times in 2 or 3 h, so prior enrichment may be shortened or even omitted after bacteria are separated and concentrated from samples. Moreover, PCR can be highly specific.

To date, the development of PCR methods for the detection of *Salmonella* and *L. monocytogenes* has focused on bacterial separation and concentration techniques from samples, as well as DNA extraction and primer selection (Rossen et al., 1991; Starbuck et al., 1992; Fluit et al., 1993a,b; Lantz et al., 1994; Soumet et al., 1994; Olsen et al., 1995; Bennett et al., 1997). In addition, various PCR product detection systems have been exploited. Agarose gel electrophoresis is a simple and widely used tool in detection of PCR products. However, it is not suitable for automation and its sensitivity needs to be improved (Rossen et al., 1991; Holmstrøm et al., 1993). Microplate assays have the advantage of automation using ELISA equipment, but the sensitivity is low (Rasmussen et al., 1994; Soumet et al., 1997). Southern hybridization has a very high sensitivity, but it is too complicated for routine use (Fluit et al., 1993b; Holmstrøm et al., 1993). Biotin, anti-digoxigenin and fluorescence detection methods are fast and highly sensitive, but their specificity is relatively low since these methods can not always distinguish non-specific PCR-amplified products (Holmstrøm et al., 1993; Cano et al., 1995). Recently, Hill et al. (1996) reported that the detection of PCR products by dot blot with radioisotope probes had the same sensitivity as Southern hybridization and could accommodate large numbers of samples. Dot blot with a radioisotope probe has been used for PCR detection of *L. monocytogenes* (Bessesen et al., 1990). While non-radioactive probes for hybridization assay have been used in many studies (Fluit et al., 1993b; Rasmussen et al., 1994; Soumet et al., 1997), very few studies, if any, have been applied to detect PCR products of *L. monocytogenes* or *Salmonella*. Therefore, the objective of the present study was to combine PCR and slot blot to develop a non-radioactive detection method for *L. monocytogenes* and *Salmonella* individually or simultaneously.

2. Materials and methods:

2.1. Bacterial cultures

L. monocytogenes (ATCC 19117) was grown in trypticase soy broth (Difco, detroit, MI, USA) supplemented with yeast extract (Difco) at 37°C and *S. typhimurium* (ATCC 14028) in nutrient broth (Difco) at 37°C. For the sensitivity test, the microorganisms grown on agar plates were inoculated in liquid media and incubated at 37°C for 6 h. The bacteria were then enumerated by the spread plate colony count method (Koch, 1981), and adjusted to 10^8 cfu/ml in culture media.

2.2. DNA extraction and PCR reactions

Bacterial DNA was extracted by mixing 90 μl of serial dilutions of the bacteria culture with 10 μl of DNA extraction buffer (0.5 M NaOH, 2.5% SDS) and heated at 90°C for 15 min. Two specific oligonucleotide primers based on the sequence of listeriolysin O gene, LM1: 5′-CCTAAGACGCCAATCGAA-3′ and LM2: 5′-AAGCGCTTGCAACTGCTC-3′ were used for *L. monocytogenes* (Border et al., 1990). For *Salmonella,* a specific pair of primers, ST11: 5′-AGCCAACCATTGCTAAATTGGCGCA-3′ and ST15: 5′-

GGTAGAAATTCCCAGCGGGTACT-3′ was used (Aabo et al., 1993).

Five microliters of bacterial DNA was subjected to PCR in 100 μl of a solution comprising 200 μM dNTP, 0.5 μM (for *L. monocytogenes*) or 0.2 μM (for *Salmonella*) primers, 10 mM Tris–HCl, pH 8.3, 0.01% gelatin, 1.7 mM $MgCl_2$, 50 mM KCl, 2.5 units Taq polymerase (Sigma, St-louis, MO, USA) and 0.7% Tween-20. For multiplex PCR, the DNA mixture subjected to PCR amplification contained 2.5 μl *of L. monocytogenes* and 2.5 μl of *S. typhimurium* DNA extracts. Both sets of primers were added in a multiplex PCR reaction system at the above respective concentrations. The reaction mixture was first denatured at 94°C for 5 min. Then 35 cycles (unless indicated otherwise) of PCR were performed (denaturation: 92°C for 30 s, annealing: 59°C for 80 s and extension: 72°C for 30 s), followed by 5 min elongation period at 72°C in a PTC-100 programmable thermal controller (MJ, Watertown, MA, USA). The PCR products were detected by both agarose gel electrophoresis and slot blot.

2.3. Detection of PCR products by slot blot hybridization

The probe for *L. monocytogenes,* 5′-NH_2-CGGATGAAATCGATAAGTAT-3′, was selected from the sequence between primers LM1 and LM2. The probe for *Salmonella* was ST14, 5′-NH_2-TTTGCGACTATCAGGTTACCGTGG-3′ (Aabo et al., 1993). These 5′-NH_2-probes were directly labeled with the reporter enzyme alkaline phosphatase through an AP-Oligonucleotide labeling kit (Boehringer Mannheim, Laval, Quebec, Canada). Briefly, 5′-NH_2-oligonucleotides were activated by the activation reagent solution. Then, the primers were purified through the ethanol precipitation procedure and mixed with activated alkaline phosphatase in the conjugation buffer. Afterwards, the AP-oligonucleotide conjugates were separated by the microconcentrator device provided by Boehringer Mannheim and stored in storage buffer (50 mM Tris–HCl, pH 7.5, 0.1% bovine serum albumin (BSA), 0.02% sodium azide) at 4°C until use.

For hybridization, 10 μl of each PCR sample was added to 400 μl of the denaturing buffer (450 mM EDTA and 0.5 N NaOH). The mixture was boiled for 10 min and then transferred to the MagnaGraph™ nylon membrane (MSI, Westborough, MA) by vacuum with a Hybri-slot™ manifold instrument (GIBCO BRL, Gaithersburg, MA, USA). DNA was bound to the nylon membrane by baking at 120°C for 20 min. The nylon membrane was subsequently prehybridized with 20 ml of hybridization solution (5 × SSC; 1% blocking reagent; 0.02% SDS) per 100 cm^2 membrane at 50°C for 40 min. The hybridization was performed at 50°C for 20 min with 0.1 μmol of AP-labeled detection probe in 2.5 ml of hybridization buffer per 100 cm^2 membrane. The membrane was then washed twice with 50 ml 2 × SSC, 0.1% SDS per 100 cm^2 membrane at room temperature for 15 min.

Detection of the hybrids on the membrane was carried out by chemiluminescence detection. The membrane was first equilibrated for 2–5 min in 20 ml of the detection buffer (0.1 M Tris–HCl, 0.1 M NaCl, pH 9.5) per 100 cm^2 membrane, then incubated with 1–2 ml of CDP-Star™ solution per 100 cm^2 membrane (Boehringer Mannheim) at room temperature for 5 min. Finally, the membrane was sealed in a hybridization bag and exposed to Biomax™ scientific imaging film (Kodak, Rochester, NY, USA) for 5–60 min, depending on the signal-noise ratio.

2.4. Immunomagnetic separation

Immunomagnetic beads (IMB) specific to *Listeria* spp. and *Salmonella* spp., respectively, were purchased from Dynal® (Oslo, Norway). The separation procedure was modified from the manufacturer's instructions. After being cultured for 6 h, bacteria were enumerated and adjusted to 10^8 cfu/ml in culture media. One-milliliter aliquots of PBS or bovine milk (pasteurized, 3.25% fat) were inoculated with serially diluted 4×10^3 to 4×10^0 cfu of *L. monocytogenes* or *S. typhimurium*. Then 100 μl of each aliquot was spread onto Bacto® Oxford Medium Base with Bacto® Modified Oxford Antimicrobic Supplement (Difco) for *L. monocytogenes* or Bacto® Hektoen Enteric Agar (Difco) for *S. typhimurium,* in order to confirm the concentration of bacterial cells added to the samples prior to IMS. The remaining 900 μl of each sample was added into a 1.5 ml microcentrifuge tube containing 20 μl of magnetic beads. After mixing by inverting the tube, the sample was incubated at room temperature for 10

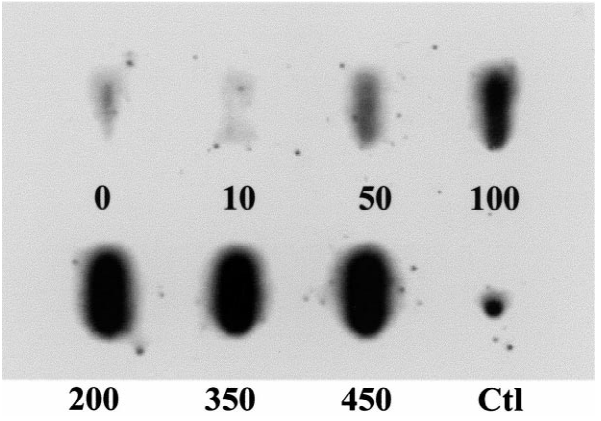

Fig. 1. Optimization of EDTA concentrations in the PCR product denaturing buffer. PCR products (35 cycles) of 10^7 cfu/ml of *S. typhimurium* were used as test samples. PCR products were denatured with 400 μl of 0.5 N NaOH containing different concentrations of EDTA in mM. For the control sample, 0.5 N NaOH containing 450 mM EDTA without PCR product was used. After denaturation, PCR products were detected by slot blot.

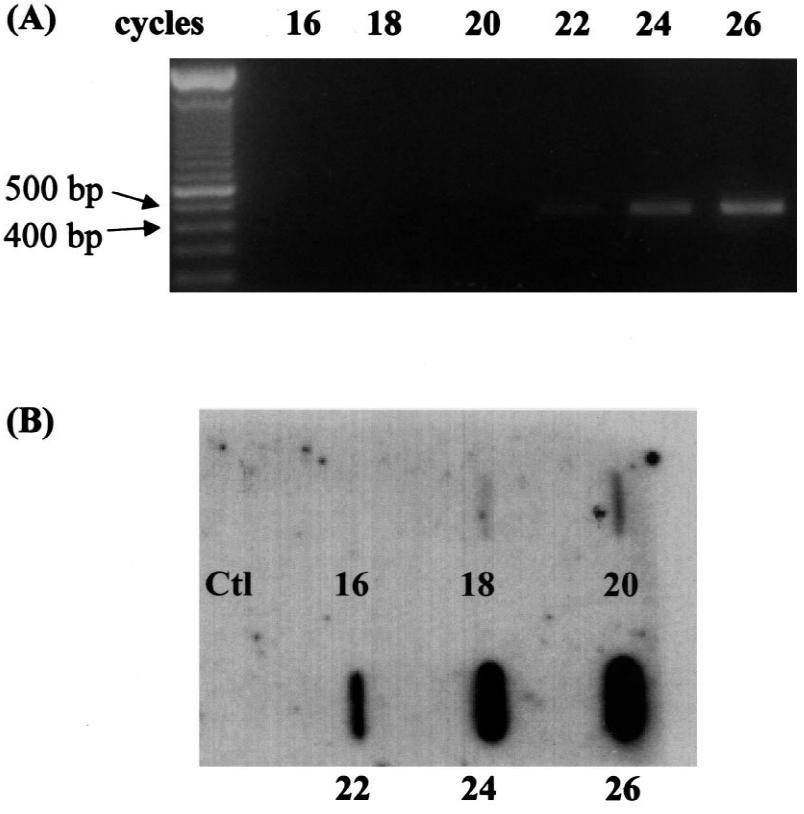

Fig. 2. Comparison of the sensitivities of slot blot and electrophoresis using PCR products from different cycles (16–26) of amplifications. DNA from 5 μl of 10^7 cfu/ml of *S. typhimurium* was used as the template. PCR products were detected by both electrophoresis (A) and slot blot (B). The denaturing buffer was used as a negative control in slot blot.

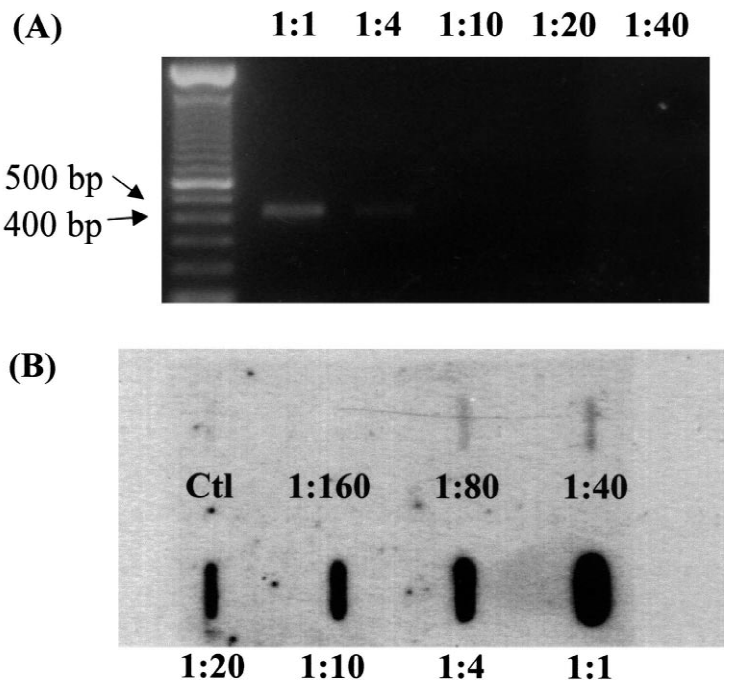

Fig. 3. Detection of PCR products after serial dilutions by electrophoresis and slot blot. After 24 cycles of amplification of 5 μl of 10^7 cfu/ml of *S. typhimurium*, PCR products were serially diluted from 1:1 to 1:160 in the PCR reaction solution and detected by both electrophoresis (A) and slot blot (B). The denaturing buffer was used as a negative control in slot blot.

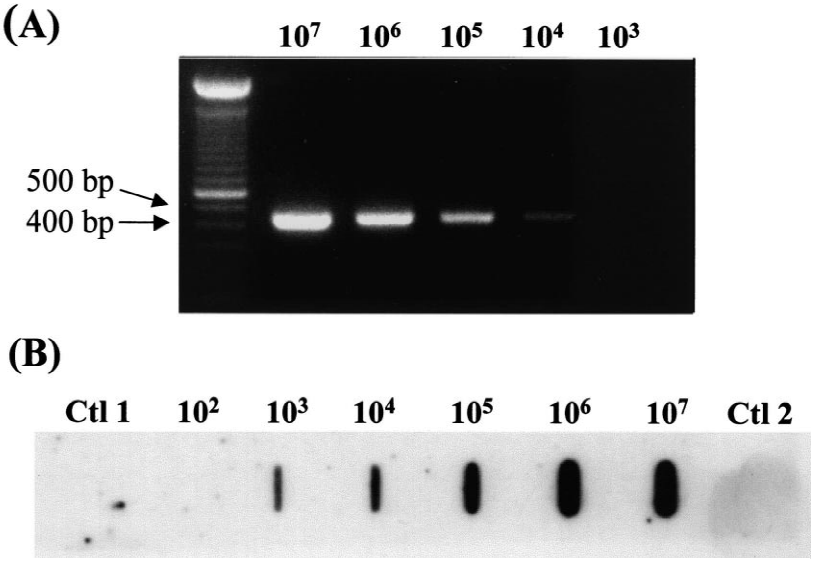

Fig. 4. Detection limit of PCR assay coupled with slot blot or electrophoresis detection for *S. typhimurium*. Cultured *S. typhimurium* were serially diluted from 10^7 to 10^2 cfu/ml in culture media. PCR products were detected by both electrophoresis (A) and slot blot (B). For slot blot, 10^7 cfu/ml of *L. monocytogenes* and the denaturing buffer were both used as negative controls (control 1 and control 2, respectively).

min with gentle agitation. The tube was then placed in a magnet separator (Biosource International, Camarillo, CA, USA) for 3 min. During this period, the rack was inverted several times in order to concentrate the beads into a pellet on the side of the tube. Then the supernatant was carefully aspirated. The magnetic beads were washed 3 times with 1 ml of PBS. After each wash, the magnetic beads were recovered with the magnet separator as described above. Finally, the magnetic particle–bacteria complex was resuspended into 100 μl of PBS. Fifty microliters of this solution was subjected to spread plate colony counts. The remaining 50 μl of solution was centrifuged at 10000*g* for 1 min, and 45 μl of supernatant was aspirated and discarded. Then 1 μl of DNA extraction buffer was added. The mixture was heated at 90°C for 15 min and then subjected to PCR detection. All the experiments in this study were repeated at least three times.

3. Results

The effect of EDTA concentration in the denaturing buffer on the binding of DNA molecules to the nylon membrane was first investigated. Various concentrations of EDTA (0, 10, 50, 100, 200, 350 and 450 mM) in 0.5 N NaOH were used. The results showed that the samples treated with 450 mM of EDTA in 0.5 N NaOH had the highest binding capacity (Fig. 1). Therefore, this concentration of denaturing buffer was used in subsequent experiments.

In order to compare the detection level of slot blot and electrophoresis, 5 μl of a 10^6 cfu/ml suspension of bacteria was used as the template. PCR products after 16, 18, 20, 22, 24 and 26 cycles of amplification were subjected to both electrophoresis and slot blot. Weak signals could be observed only from PCR products after 22 cycles by electrophoresis, whereas PCR products after 18 cycles could be detected by slot blot (Fig. 2). To further quantify the detection limit of the slot blot in comparison with that of electrophoresis, serial dilutions of PCR products from 1:1 to 1:160 after 24 cycles of PCR were subjected to both electrophoresis and slot blot. The PCR product at the 1:4 dilution could be detected by electrophoresis, whereas the 1:80 PCR product was detectable by slot blot, i.e. the slot blot was about 20 times more sensitive than electrophoresis (Fig. 3).

In order to investigate the detection limit of the PCR assay coupled with the slot blot for the detection for *S. typhimurium* and *L. monocytogenes* in

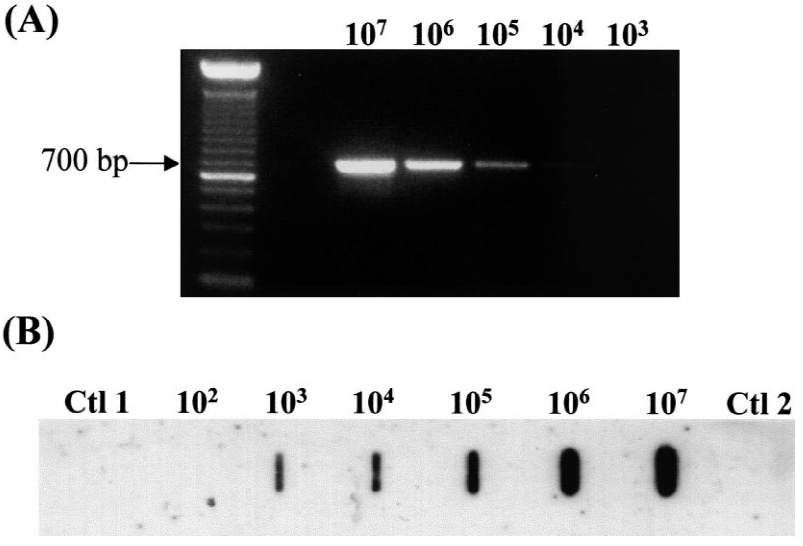

Fig. 5. Detection limit of PCR assay coupled with slot blot or electrophoresis detection for *L. monocytogenes*. Cultured *L. monocytogenes* were serially diluted from 10^7 to 10^2 cfu/ml in culture media. PCR products were detected by both electrophoresis (A) and slot blot (B). For slot blot, 10^7 cfu/ml of *S. typhimurium* and the denaturing buffer were both used as negative controls (control 1 and control 2, respectively).

pure culture, the bacteria were serially diluted from 10^7 to 10^2 cfu/ml with culture media. After PCR amplification of bacterial DNA extracts, 10 μl of each PCR product was subjected to both electrophoresis and slot blot. As shown in Figs. 4 and 5, cell suspension at a concentration of 10^4 cfu/ml could be faintly detected by electrophoresis for both bacteria. This corresponds to 50 bacteria in each PCR reaction. On the other hand, the detection limit for the slot blot was 10^3 cfu/ml, and corresponding to 5 bacteria in each PCR reaction, i.e. the slot blot was 10 times more sensitive than electrophoresis.

To further investigate the potential of this PCR assay coupled with slot blot for the detection of *S. typhimurium* and *L. monocytogenes* in food samples, inoculated milk samples containing 4×10^4 to 4×10^0 bacteria were subjected to immunomagnetic separation (IMS). After PCR amplification of bacterial DNA extracted from bacteria-coated IMB, PCR products were subjected to both electrophoresis and slot blot. The experiment was repeated at least three times. For *L. monocytogenes*, detection limits of 4×10^2 and 4×10^1 cfu/ml were obtained by electrophoresis and slot blot, respectively (Fig. 6). Similar results were obtained for the detection of *S. typhimurium* (data not shown).

Simultaneous detection of these two pathogens was also carried out by multiplex PCR in combination with the slot blot. As shown in Fig. 7, both bacteria could be detected within a single reaction when they were present at a concentration as low as 10^3 cfu/ml.

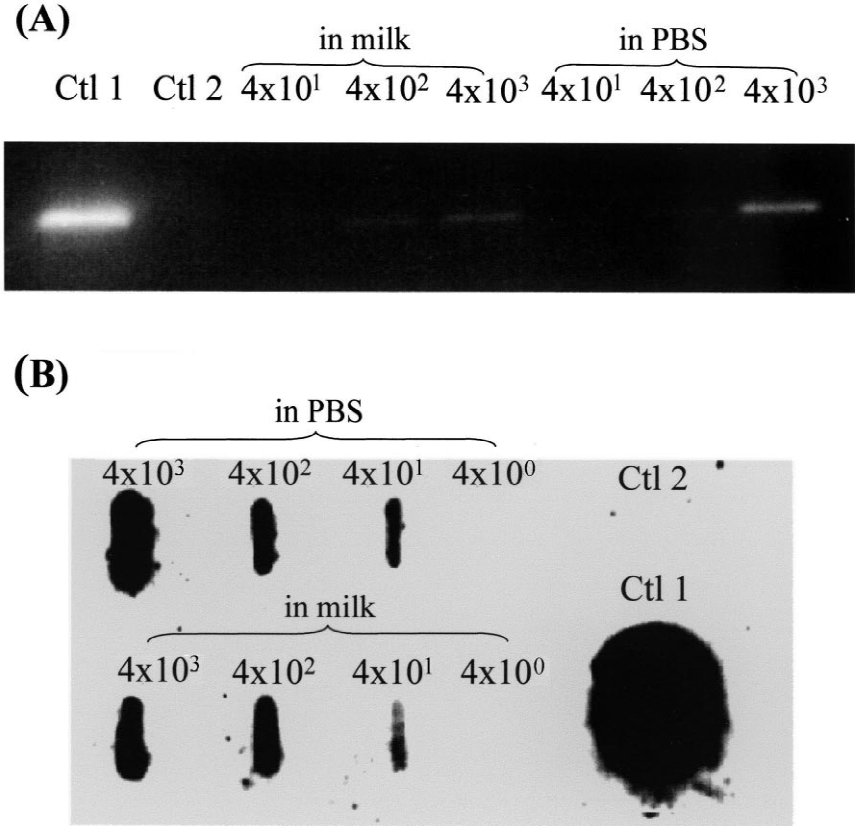

Fig. 6. Detection of bacteria-bound IMB by PCR coupled with electrophoresis detection or slot blot. Cultured *L. monocytogenes* were serially diluted from 4×10^3 to 4×10^0 cfu/ml in PBS or milk and subjected to IMS. After PCR amplification of bacterial DNA extracted from bacteria-coated IMB, PCR products were detected by electrophoresis (A) or slot blot (B). PCR products (35 cycles) of 10^7 cfu/ml of *L. monocytogenes* were used as control 1. Ten microliters of IMB without IMS was used as control 2 for slot blot detection.

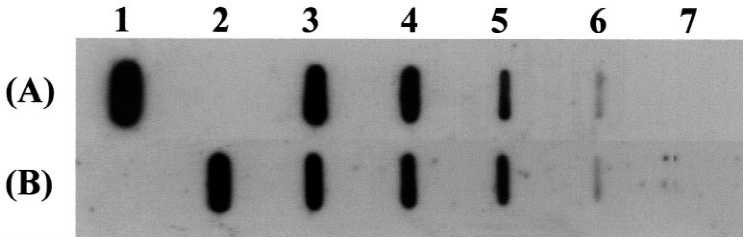

Fig. 7. Simultaneous detection of *Salmonella* and *L. monocytogenes* by PCR assay coupled with slot-blot detection. Cultured *Salmonella* and *L. monocytogenes* were serially diluted from 10^6 to 10^2 cfu/ml in culture media. After multiplex PCR amplification, PCR products were detected by slot blot with the probe for *L. monocytogenes* (A) or the probe for *S. typhimurium* (B). Slot 1 and slot 2 contain PCR products from 10^6 cfu of *L. monocytogenes* per ml or from 10^6 cfu of *Salmonella* per ml, respectively. Slots 3–7 contain PCR products from both *L. monocytogenes* and *Salmonella*. Slot 3: 10^6 cfu/ml from each bacterium; slot 4: 10^5 cfu/ml; slot 5: 10^4 cfu/ml; slot 6: 10^3 cfu/ml; and slot 7: 10^2 cfu/ml.

4. Discussion

In the present study, the PCR method coupled with slot blot hybridization using non-radioactive probes was successfully adopted to detect both *Salmonella* and *L. monocytogenes* in culture media, with a detection limit of 10^3 cfu/ml. This detection limit is about 10 times more sensitive than that of PCR coupled with electrophoresis. Combined with IMS to separate and concentrate the bacteria from samples, the detection limit was 40 cfu/ml of bacteria from milk samples. This method could detect a large number of samples within 7 h. Moreover, the method permitted simultaneous detection of these two bacteria.

The detection limit of 10^3 cfu/ml of sample by PCR in combination with a slot blot corresponds to 5 bacteria in the PCR reaction. For DNA extraction, 10 μl of DNA extraction solution (0.5 M NaOH with 2.5% SDS) was mixed with 90 μl of bacterial culture. In a previous study (Rossen et al., 1991), 5 μl of the culture were mixed with 50 μl of 0.05 M NaOH and 0.25% SDS to extract the bacterial DNA. Therefore, although 5 bacterial cells in the PCR reaction were detected in their study, the detection limit was only 10^4 cfu/ml in the starting suspensions. The PCR products of 5 live bacteria could be detected by electrophoresis in Rossen's study. However, 50 bacterial cells were needed in the PCR reaction for detection by electrophoresis in our study. In another study, the detection limit by electrophoresis was also 50 bacterial cells (Holmstrøm et al., 1993). The different sensitivities of the PCR method in combination with electrophoresis in Rossen's study and ours may be due to different PCR conditions and/or the presence of dead cells in culture. Since DNA from both viable and nonviable cells may be amplified by PCR, the bacteria were cultured in the liquid media for only 6 h in our study to ensure their being in the logarithmic phase of growth (data not shown). This helped to minimize the error caused by the presence of dead cells in culture.

Our results showed that EDTA greatly enhanced the capability of DNA to bind to the nylon membrane. While PCR products without EDTA in the denaturing buffer were detected by slot blot, adding EDTA into the denaturing buffer improved DNA binding to the membrane and detection by slot blot. How EDTA interacts with chemical(s) in our PCR reaction solution and consequently affects the binding of DNA to the membrane is still unclear. One possibility is that EDTA could remove divalent cations which otherwise complex with the negatively charged DNA molecules and prevent them from interacting with the positively charged nylon membrane. It has been reported that magnesium ions in the PCR reaction solution could inhibit viral and plasmid DNA binding to the nylon membrane (Kube and Srivastava, 1997). Understanding the mechanism of adsorption of bacterial DNA to the membrane may be helpful to enhance the binding of DNA to membranes and therefore increase the sensitivity of detection.

In the present study, probes were directly labeled with the reporter enzyme alkaline phosphatase and

only one step of detection was required. In comparison with digoxigenin-labeled (Fluit et al., 1993b) or biotinylated probes (Soumet et al., 1997), which require two detection steps, an alkaline phosphatase-labeled probe can reduce the time and labor of detection with the same level of detection. The primers used for DNA amplification are specific for both bacterial species as reported previously (Border et al., 1990; Aabo et al., 1993). Few if any other DNA molecules will be amplified. Due to the specific probes used in this study, this minimized the chance for non-specific detection.

The major advantages of combining PCR with the slot blot detection in this study is improvement of overall specificity, sensitivity and efficiency when compared with other PCR product detection methods. Detection probes corresponding to the sequences of PCR products were used in the slot blot technique. This avoided non-specific detection of PCR products, which may be present in the biotinylated and anti-digoxigenin (Holmstrøm et al., 1993) and fluorescence detection methods (Cano et al., 1995). In addition, five bacterial cells in the PCR reaction could be detected in the present study. This sensitivity is higher than that of the microplate assays, which require 50–1000 templates in the PCR reaction (Rasmussen et al., 1994; Soumet et al., 1997). Using Southern hybridization for the detection of PCR products, the sensitivity is similar to that of the slot blot technique. However, the processing of PCR products by Southern hybridization requires 5 h to 1 day and is much more laborious (Holmstrøm et al., 1993; Hill et al., 1996). With our slot blot instrument, 24 samples can be applied simultaneously. The whole process for this slot blot assay is about 3 h. Moreover, our method avoids the use of hazardous material such as ethidium bromide in conventional agarose gel electrophoresis and radioactive materials in radioisotope-based hybridization methods. Therefore, the detection of the PCR product by slot blot is specific, sensitive, safe, rapid and suitable to handle a large number of samples.

Many methods that can separate and concentrate bacteria from various samples have been developed (Starbuck et al., 1992; Fluit et al., 1993a,b; Lantz et al., 1994; Bennett et al., 1997). Among these methods, IMS has been most widely studied. Recently, several pathogens such as *Listeria* spp. (Avoyne et al., 1997) and *Mycobacterium paratuberculosis* (Grant et al., 1998) have been separated by IMS with high recovery rates directly from various samples containing very low numbers of pathogens. For the present PCR system coupled with slot blot detection in food samples, IMS was used to separate and concentrate bacteria from inoculated milk samples prior to PCR detection. The same sensitivity of 40 cfu/ml of bacteria was obtained with PCR detection after IMS from PBS and milk. This suggests that IMS not only enabled recovery of bacteria from milk samples, but also separated the bacteria from inhibitory substances in the milk, which may greatly reduce the sensitivity of PCR detection (Wilson, 1997). IMS has not been applied to any other kind of food samples in this study and may need further investigation. A previous study has shown that IMS could separate *Salmonella* in vacuum-packed ham samples, soft cheese and whole milk powder (Skjerve and Olsvik, 1991). Therefore, combined with IMS or other methods of bacterial separation and concentration, the PCR coupled with the slot blot detection method reported in the present study is promising for the rapid detection of *Salmonella* and *L. monocytogenes* in foods.

The simultaneous detection of several bacteria with multiplex PCR is more time-efficient and cost-effective (Brasher et al., 1998). A single PCR cycling program for both *Salmonella* and *L. monocytogenes* was developed in our study. High yields of PCR products were obtained for both bacteria with this PCR program. Using the same slot blot conditions, both bacteria could be simultaneously detected at a level of 10^3 cfu/ml for each bacterial sample. Some previous studies have shown that three or four pathogens could be successfully detected by multiplex PCR (Hendolin et al., 1997; Brasher et al., 1998; Garcia et al., 1998). However, it is also worthwhile to point out that high numbers of one bacterial species may reduce the sensitivity of the PCR assay for the other pathogens (Rossen et al., 1991).

Acknowledgements

The authors express their gratitude to Dr. L. Jin for his critical review. This work was supported by a grant from Conseil de Recherche en Pêche et en Agroalimentaire du Québec (CORPAQ) to X. Zhao.

References

Aabo, S., Rossen, L., Rasmussen, O.F., Sorensen, P.D., Olsen, J.E., 1993. *Salmonella* identification by the polymerase chain reaction. Mol. Cell. Probes 7, 171–178.

Avoyne, C., Butin, M., Delaval, J., Bind, J., 1997. Detection of *listeria* spp. in food samples by immunomagnetic capture: ListerScreen method. J. Food Protect. 60, 377–384.

Bennett, A.R., Davids, F.G.C., Vlahodimou, S., Banks, J.G., Betts, R.P., 1997. The use of bacteriophage-based systems for the separation and concentration of *Salmonella*. J. Appl. Microbiol. 83, 259–265.

Bessesen, M.T., Luo, Q., Rotbart, H.A., Blaser, M.J., Ellison, R.T., 1990. Detection of *Listeria monocytogenes* by using the polymerase chain reaction. Appl. Environ. Microbiol. 56, 2930–2932.

Border, P.M., Howard, J.J., Plastow, G.S., Siggens, K.W., 1990. Detection of *Listeria* species and *Listeria monocytogenes* using polymerase chain reaction. Lett. Appl. Microbiol. 11, 158–162.

Brasher, C.W., DePaola, A., Jones, D.D., Bej, A.K., 1998. Detection of microbial pathogens in shellfish with multiplex PCR. Curr. Microbiol. 37, 101–107.

Cano, R.J., Norton, D.M., Inzunza, A.E., Sanchez, J.G., Oste, C., 1995. Polymerase chain reaction assay coupled with fluorescence detection on microwell plates for *Listeria monocytogenes* in foods. J. Food Protect. 58, 614–620.

Catteau, M., 1995. The genus *Listeria*. In: Bourgeols, C.M., Leveau, J.Y. (Eds.), Microbiological Control for Foods and Agricultural Products, VCH publishers, New York, pp. 373–382.

Clarke, R.C., Gyles, C.L., 1993. Salmonella. In: Gyles, C.L., Thoen, C.O. (Eds.), Pathogenesis of Bacterial Infections in Animals, 2nd Edition, Iowa State University Press, Ames, pp. 133–153.

Czuprynski, C.J., 1993. Listeria. In: Gyles, C.L., Thoen, C.O. (Eds.), Pathogenesis of Bacterial Infections in Animals, 2nd Edition, Iowa State University Press, Ames, pp. 70–79.

Dalton, C.B., Austin, C.C., Sobel, J., Hayes, P.S., Bibb, W.F., Graves, L.M., Swaminathan, B., Proctor, M.E., Griffin, P.M., 1997. An outbreak of gastroenteritis and fever due to *Listeria monocytogenes* in milk. N. Engl. J. Med. 336, 100–105.

Fluit, A.C., Torensma, R., Visser, M.J.C., Aarsman, C.J.M., Poppelier, M.J.J.G., Keller, B.H.I., Klapwijk, P., Verhoef, J., 1993a. Detection of *Listeria monocytogenes* in cheese with the magnetic immuno-polymerase chain reaction assay. Appl. Environ. Microbiol. 59, 1289–1293.

Fluit, A.C., Widjojoatmodjo, M.N., Box, A.T.A., Torensma, R., Verhoef, J., 1993b. Rapid detection of *Salmonellae* in poultry with the magnetic immuno-polymerase chain reaction assay. Appl. Environ. Microbiol. 59, 1342–1346.

Fung, D.Y., 1995. Rapid methods and automation in food microbiology: an update. In: Bourgeols, C.M., Leveau, J.Y. (Eds.), Microbiological Control for Foods and Agricultural Products, 1, VCH publishers, New York, pp. 3–22.

Garcia, L., Tercero, J.C., Legido, B., Ramos, J.A., Alemany, J., Sanz, M., 1998. Rapid detection of *Actinobacillus actinomycetemcomitans*, *Prevotella intermedia* and *Porphyromona gingivalis* by multiplex PCR. J. Periodontal Res. 33, 59–64.

Gledel, J., Corbion, B., 1995. The genus *Salmonella*. In: Bourgeols, C.M., Leveau, J.Y. (Eds.), Microbiological Control for Foods and Agricultural Products, VCH publishers, New York, pp. 309–324.

Glynn, M.K., Bopp, C., Dewitt, W., Dabney, P., Mokhtar, M., Angulo, F.J., 1998. Emergence of multidrug-resistant *Salmonella* enterica serotype *typhimurium* DT104 infections in the United States. N. Engl. J. Med. 338, 1333–1338.

Grant, I.R., Ball, H.J., Rowe, M.T., 1998. Isolation of *Mycobacterium paratuberculosis* from milk by immunomagnetic separation. Appl. Environ. Microbiol. 64, 3153–3158.

Helmick, C.G., Griffin, P.M., Addiss, D.G., Tauxc, R.V., Juranck, D.D., 1994. Infectious diarrheas. In: Everhart, J.E. (Ed.), Digestive Diseases in the United States: Epidemiology and Impact, 3, Government Printing Office, Washington, DC, pp. 83–124.

Hendolin, P.H., Markkanen, A., Ylikoski, J., Wahlfors, J.J., 1997. Use of multiplex PCR for simultaneous detection of four bacterial species in middle ear effusions. J. Clin. Microbiol. 35, 2854–2858.

Hill, J.M., Halford, W.P., Wen, R., Engel, L.S., 1996. Quantitative analysis of polymerase chain reaction products by dot blot. Nucleic Acid Res. 235, 44–48.

Holmstrøm, K., Rossen, L., Rasmussen, O.F., 1993. A highly sensitive and fast nonradioactive methods for detection of polymerase chain reaction products. Anal. Biochem. 209, 278–283.

Koch, A.L., 1981. Growth measurement. In: Gerhardt, P., Murray, R.G.E., Costilow, R.N., Nester, E.W., Wood, W.A., Krieg, N.R., Phillips, G.B. (Eds.), Manual of Methods for General Bacteriology, American Society for Microbiology, Washington, DC, pp. 179–207.

Kube, D.M., Srivastava, A., 1997. Quantitative DNA slot blot analysis: inhibition of DNA binding to membrane by magnesium ions. Nucleic Acids Res. 25, 3375–3376.

Lantz, P.G., Tjerneld, F., Borch, E., Hahn-Hagerdal, B., Radstrom, P., 1994. Enhanced sensitivity in PCR detection of *Listeria monocytogenes* in soft cheese through use of an aqueous two-phase system as a sample preparation method. Appl. Environ. Microbiol. 60, 3416–3418.

Olsen, J.E., Aabo, S., Hill, W., Notermans, S., Wernars, K., Granum, P.E., Popovic, T., Rasmussen, H.N., Olsvik, F., 1995. Probes and polymerase chain reaction for detection of foodborne bacterial pathogens. Int. J. Food Microbiol. 28, 1–78.

Rasmussen, S.R., Rasmussen, H.B., Larsen, M.R., Hoff-Jorgensen, R., Cano, R.J., 1994. Combined polymerase chain reaction–hybridization microplate assay used to detect bovine leukemia virus and *Salmonella*. Clin. Chem. 40, 200–205.

Riedo, F.X., Pinner, R.W., Tosca, M.L., Cartter, M.L., Graves, L.M., Reeves, M.W., Weaver, R.E., Plikaytis, B.D., Broome, C.V., 1994. A point-source foodborne listeriosis outbreak: documented incubation period and possible mild illness. J. Infect. Dis. 170, 693–696.

Rossen, L., Holmstrøm, K., Olsen, J.E., Rasmussen, O.F., 1991. A rapid polymerase chain reaction (PCR)-based assay for the identification of *L. monocytogenes* in food samples. Int. J. Food Microbiol. 14, 145–152.

Skjerve, E., Olsvik, F., 1991. Immunomagnetic separation of *Salmonella* from foods. Int. J. Food Microbiol. 14, 11–18.

Soumet, C., Ermel, G., Fach, P., Colin, P., 1994. Evaluation of different DNA extraction procedures for the detection of *Salmonella* from chicken products by polymerase chain reaction. Lett. Appl. Microbiol. 19, 294–298.

Soumet, C., Ermel, G., Salvat, G., Colin, P., 1997. Detection of *Salmonella* spp. in food products by polymerase chain reaction and hybridization assay in microplate format. Lett. Appl. Microbiol. 24, 113–116.

Starbuck, M.A.B., Hill, P.J., Stewart, G.S.A.B., 1992. Ultra sensitive detection of *Listeria monocytogenes* in milk by the polymerase chain reaction (PCR). Lett. Appl. Microbiol. 15, 248–252.

Wilson, I.G., 1997. Inhibition and facilitation of nucleic acid amplification. Appl. Environ. Microbiol. 63, 3741–3751.