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Simultaneous detection of *Salmonella* spp and *Escherichia coli* O157:H7 by multiplex PCR

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Contamination of foods with pathogens such as *Escherichia coli* O157:H7 and *Salmonella* is a major concern worldwide and rapid, sensitive, and reliable methods are needed for detection of these organisms. Since these pathogens can contaminate similar foods and other types of samples, a multiplex polymerase chain reduction (PCR) was designed to allow simultaneous detection of both *E. coli* O157:H7 and *Salmonella* spp directly from enrichment cultures. Samples of apple cider, beef carcass wash water, ground beef, and bovine feces were inoculated with both *E. coli* O157:H7 and *S. typhimurium* at various bacterial levels. Following enrichment culturing for 20–24 h at 37°C in modified EC broth or buffered peptone water both containing novobiocin, the samples were subjected to a DNA extraction technique or to immunomagnetic separation then tested by the multiplex PCR assay. Four pairs of primers were employed in the PCR: primers for amplification of *E. coli* O157:H7 *eaeA*, $stx_{1/2}$ and plasmid sequences and for amplification of a portion of the *Salmonella invA* gene. Four fragments of the expected sizes were amplified in a single reaction and visualized following agarose gel electrophoresis in all the samples inoculated with $\leq$ 1 CFU g^{-1} or ml^{-1}. Results can be obtained in approximately 30 h. The multiplex PCR is a potentially powerful technique for rapid and sensitive co-detection of both pathogens in foods and other types of samples.

Keywords: immunomagnetic separation; bovine feces; carcass wash water; apple cider; ground beef

Introduction

E. coli O157:H7 and *Salmonella* are food-borne pathogens of major concern in the United States and worldwide. Estimates indicate that there are approximately 10 000 to 20 000 cases of illness caused by *E. coli* O157:H7 yearly with 200–500 deaths [4]. *Salmonella* is responsible for the largest number of bacterial food-borne disease outbreaks in the US [1]. Cattle, sheep and deer are carriers of *E. coli* O157:H7 and *S. typhimurium* is found in the intestinal tracts of warm-blooded animals including cattle. During the beef-slaughtering process, the pathogens can be accidentally introduced onto carcass surfaces, in particular during hide removal and evisceration. Ground beef and other foods of bovine origin have been identified as sources of infection in many food-borne disease outbreaks and sporadic cases associated with *E. coli* O157:H7 and *S. typhimurium* infection [5,6,19]. It is estimated that the prevalence of *Salmonella* in beef ranges from 1% for raw beef carcasses to 5–7% for ground beef [5]. Both *E. coli* O157:H7 and *Salmonella* can be extremely tolerant to acidic environments and disease outbreaks have been associated with consumption of unpasteurized orange juice and apple cider [14]. The infectious dose of both organisms is believed to be relatively low [15,18], in particular in the very young, the elderly and the immunocompromised.

Several *E. coli* O157:H7 virulence determinants involved in pathogenicity include: production of stx_1 and/or stx_2 which inhibit protein synthesis in host cells and *eae* genes which are involved in the formation of attaching and effacing lesions on intestinal cells [7,19]. A large plasmid harbored by virtually all *E. coli* O157:H7 strains has been found to encode for a hemolysin which may also play a role in virulence [17]. Salmonellae adhere to and invade mammalian cells and an *inv* locus, located on a pathogenicity island (SPI 1), is required for bacterial internalization into cultured cells. *Inv* genes are highly conserved in the genus *Salmonella* [9].

Conventional cultural methods for detection and identification of *Salmonella* spp or *E. coli* O157:H7 in foods are generally very cumbersome and time-consuming. Furthermore, the pathogens are often present in very low numbers among a background of indigenous microflora, thus rendering recovery of target organisms difficult. Methods should be capable of detecting one viable target organism in 25 g of food in a very short period of time. Rapid and sensitive assays of high specificity are needed for detection of pathogenic bacteria in foods and other types of samples. PCR-based methods have the potential to allow for rapid, sensitive detection of food-borne pathogens. Since PCR can target unique genetic sequences such as virulence genes of microorganisms, it also has the advantage of potentially being an extremely specific assay.

We previously described a multiplex PCR for identification of *E. coli* O157:H7 colonies grown on selective and differential medium [7]. Since both *E. coli* O157:H7 and *Salmonella* spp are pathogens of major concern and can

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be found as contaminants in similar types of samples, the objective of the present study was to use a multiplex PCR approach to detect both organisms simultaneously directly from enrichment cultures of ground beef, bovine feces, beef carcass wash water and unpasteurized apple cider with minimum sample pretreatment.

Materials and methods

Bacterial strains

E. coli O157:H7 B1409 (produces stx_2) was obtained from the Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA and *S. typhimurium* 14028 was obtained from the American Type Culture Collection (ATCC), Rockville, MD, USA. The bacterial strains listed in Table 1 were from our laboratory culture collection and were from various sources including CDC and ATCC or were human, food or animal isolates. The salmonellae, with the exception of *S. arizonae*, *S. poona*, and *S. enteriditis*, were isolated in our laboratory from environmental samples contaminated with swine fecal material. The cultures were maintained at 4°C on tryptic soy agar (Difco Laboratories, Detroit, MI, USA) slants. A loopful of colonies from each of the slants was inoculated separately into flasks containing brain heart infusion broth (Difco). The cultures were grown overnight at 37°C with aeration, then diluted in 0.1% peptone (Difco) to obtain *ca* 10^3, 10^2, 10^1, 5×10^0 and 1×10^0 CFU ml^{-1}. The actual number of bacteria in the dilutions was determined by plating onto MacConkey sorbitol agar, incubating the plates overnight at 37°C, then manually counting the colonies. Prior to enrichment culturing, 2.5 ml of the bacterial dilutions was added to each of the samples and the samples were processed as described below.

Table 1 PCR results of bacterial strains tested for *invA* gene sequence

Bacteria	Number of strains tested	PCR result
Citrobacter braakii	1	–[a]
Citrobacter freundii	2	—
Escherichia coli	10	—
Listeria ivanovii	1	—
Listeria monocytogenes	3	—
Listeria welshimeri	1	—
Salmonella		
S. amager	1	+[b]
S. anatum	1	+
S. arizonae	1	+
S. brandenburg	1	+
S. derby/virchow	1	+
S. dublin	1	+
S. enteritidis	3	+
S. heidelberg	1	+
S. infantis	1	+
S. mbandaka	1	+
S. muenster	1	+
S. newbrunswick	1	+
S. newport	1	+
S. poona	1	+
S. sandiego	1	+
S. senftenberg	2	+
S. typhimurium	22	+
S. worthington	1	+
Serratia marcescens	1	–
Shewanella putrefaciens	1	–
Shigella boydii	1	–
Shigella dysenteriae	2	–
Shigella flexneri	4	–
Shigella sonnei	2	–
Vibrio parahaemolyticus	1	–
Yersinia enterocolitica	7	–

[a]Negative PCR result.
[b]Positive PCR result.

Samples tested

Lean ground beef was purchased from a local supermarket and used on the same day. Fresh-pressed unpasteurized apple cider was purchased from a local college and stored at −20°C prior to use. Bovine feces was obtained from the same college. The top portion of bovine feces freshly deposited on the ground was collected, placed into plastic bags and stored at −20°C prior to use. Carcass wash water was obtained from a local beef slaughter plant. After carcasses were split and washed (CHAD washing system, 250 psi spray pressure), the water that was shed from the split carcasses was collected in sterile plastic containers with the aid of a large funnel. The wash water was refrigerated for approximately 24 h, then divided into smaller portions and frozen at −20°C prior to use.

Sample processing and enrichment

The procedure employed for inoculation and processing of samples is shown in Figure 1. Beef carcass wash water was divided into 300-ml portions and *E. coli* O157:H7 and *Salmonella* (2.5 ml of the bacterial dilutions) were added at similar levels to each sample. To the negative control samples, 2.5 ml of sterile 0.1% peptone was added. The wash water was filtered through 2-μm pore size glass fiber prefilters (Fisher Scientific, Pittsburgh, PA, USA) placed in porcelain Büchner filter funnels. This was followed by filtering the filtrate through 0.2-μm pore size Nalgene® nylon filters (A Daigger & Co, Wheeling, IL, USA). The membranes were then cut from the filters using a sterile dissecting scalpel and, using sterile tweezers, were placed into 500-ml Erlenmeyer flasks containing 245 ml of mEC + novobiocin (0.02 mg ml^{-1}) (mEC + n) [13].

Twenty-five grams of ground beef were added to 225 ml of mEC + n or to 225 ml of buffered peptone water (Difco) containing 0.02 mg ml^{-1} of novobiocin (BPW + n). Twenty-five milliliters of apple cider were added to 225 ml of mEC + n and 5 g of bovine feces was placed into flasks containing either 245 ml of mEC + n or BPW + n. Dilutions of *E. coli* O157:H7 and *S. typhimurium* (2.5 ml) were then added to each flask, then the samples were incubated at 37°C for 20–24 h with aeration at 150 rpm. Ten milliliters of the enrichment cultures were centrifuged at $4100 \times g$ for 10 min and the cell pellets were resuspended in 2 ml of BPW. With the bovine fecal samples, 11 ml of the enrichment cultures was centrifuged at $100 \times g$ for 5 min to remove large debris, then 10 ml of the supernatant was centrifuged again for 10 min at $4100 \times g$. The pellet was then suspended in 2 ml of BPW. The ground beef and bovine fecal enrichment cultures were also plated onto XLT4 (Difco), BG Sulfa (containing 15 mg L^{-1} novobiocin; Difco), Rainbow (containing 50 mg L^{-1} novobiocin;

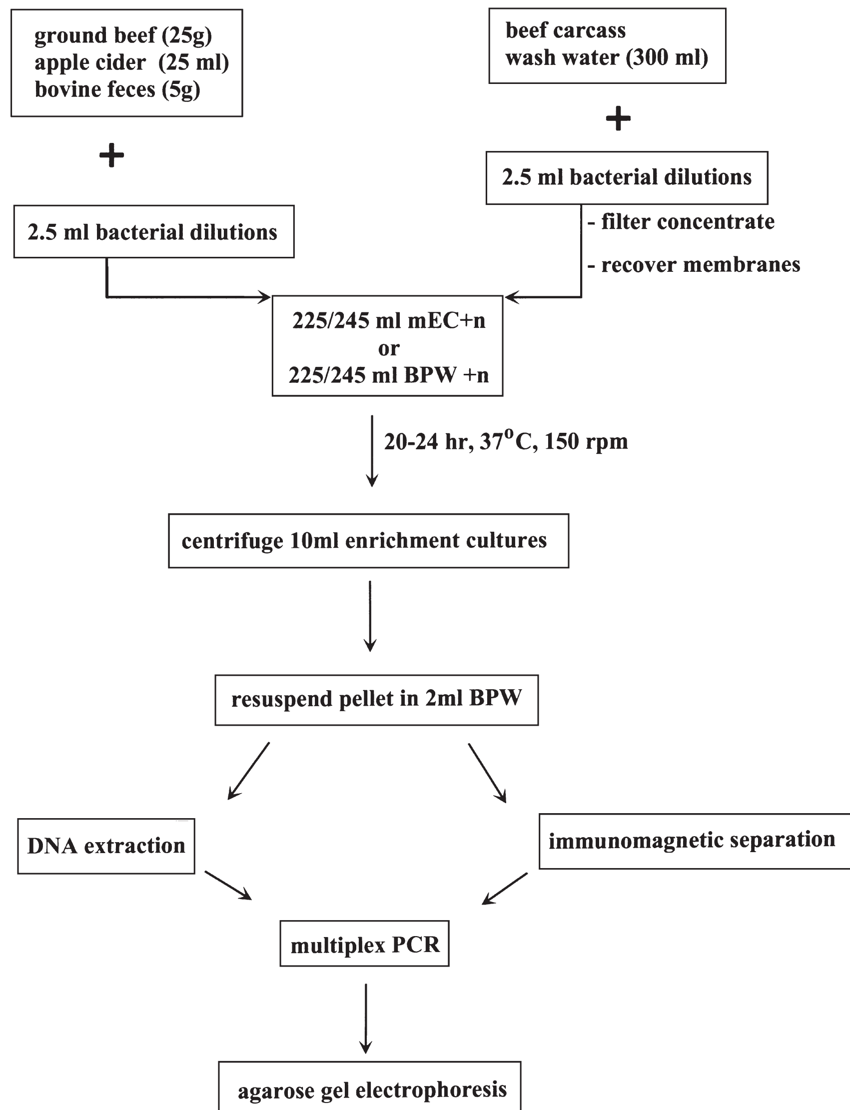

Figure 1 Flow chart showing procedure used to process and test samples by multiplex PCR.

Biolog, Hayward, CA, USA) and CT-SMAC [11] agars to determine if *E. coli* O157:H7 and *S. typhimurium* colonies could easily be recovered and identified using these media.

DNA extraction

Two procedures for isolation and concentration of bacterial DNA in the samples were compared. Five hundred microliters of the resuspended cell pellets were heated for 20 min at 100°C and the DNA was then extracted with a 1:1 mixture of a phenol-chloroform solution using Phase Lock Gel™ (I Heavy; 5 Prime → 3 Prime, Boulder, CO, USA). Following ethanol precipitation of the nucleic acids, the pellets were washed once with 70% ethanol, dried, and resuspended in 50 μl of sterile distilled water. Alternatively, the High Pure PCR Template Preparation Kit (Boehringer Mannheim, Indianapolis, IN, USA) was also employed to isolate nucleic acids from PCR inhibitory components in the samples. The resuspended cell pellets (500 μl) were centrifuged for 2 min at $16\,000 \times g$ and suspended again in 200 μl of phosphate-buffered saline, pH 7.4. Thirty microliters of lysozyme (10 mg ml^{-1} in 100 mM Tris, pH 8.0) were added and the samples were incubated for 15 min in a 37°C water bath. DNA was isolated from the cell lysate according to the instructions provided by the manufacturer of the kit.

Immunomagnetic separation

E. coli O157:H7 and *S. typhimurium* were recovered from the resuspended cell pellets from the ground beef and bovine fecal enrichments by immunomagnetic separation (IMS). One milliliter of sample was mixed with 20 μl of Dynabeads® anti-*Salmonella* (Dynal, Lake Success, NY, USA) and 20 μl of Dynabeads® anti-*E. coli* O157 in 1.5-ml microcentrifuge tubes. The tubes were placed on a Roto-Torque (Cole Parmer Instruments, Chicago, IL, USA) and rotated gently at *ca* 14 rpm for 30 min at room temperature. The beads were concentrated onto the side of the tubes using a Dynal MPC-M magnetic device and the supernatant phase was discarded. The beads were washed twice with phosphate-buffered saline, pH 7.4, containing 0.05%

Tween 20, suspended in 40 μl of sterile distilled water, then heated to 99°C for 10 min in a Perkin Elmer GeneAmp PCR System 9600 thermal cycler (Perkin Elmer Applied Biosystems, Foster City, CA, USA) to lyse the bacteria.

Multiplex PCR and analysis of amplification products

The oligonucleotide primers used in the multiplex PCR and the sizes of the expected PCR products are listed in Table 2. To 5-μl portions of the DNA recovered by the DNA extraction procedures and 5 μl of supernatant recovered from the bead mixture following IMS after lysis, 10 μl of GeneReleaser™, a reagent which allows amplification of unpurified DNA (BioVentures, Murfreesboro, TN, USA), was added. The samples were subjected to the thermocycling program recommended by the manufacturer of GeneReleaser™ and subsequently, 35 μl of a PCR mixture was added to each tube. The PCR reaction mixture consisted of 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 3.0 mM $MgCl_2$, 0.1% Triton X-100, 400 μM (each) of the four deoxynucleoside triphosphates (dNTPs) (Gibco BRL Life Technologies, Gaithersburg, MD, USA), 2.5 U *Taq* DNA polymerase (Gibco BRL), 0.50 μM of primers MFS1F, MFS1R, MK1 and MK2 and 0.25 μM of primers AE22, AE20-2, INVAF and INVAR. The reaction mixtures were heated at 94°C for 2 min and subjected to 38 cycles of denaturation at 94°C for 20 s, annealing at 53°C for 1 min, and extension at 72°C for 1 min, with an additional 10-min extension at 72°C. Products of the multiplex PCR were subjected to electrophoresis on 1.5% agarose gels which were then stained with ethidium bromide, examined using a UV transilluminator and photographed.

Results

Various serovars of *Salmonella* and a number of other bacteria were screened by PCR to determine the specificity of the *invA* primers. An amplification product of the expected size was observed with all the *Salmonella* (including 14 *S. typhimurium* DT104 isolates) strains tested; however, no PCR product was generated with amplification of DNA from *E. coli* (including four *E. coli* O157:H7 strains), *Citrobacter*, *Listeria*, *Shewanella*, *Shigella*, *Vibrio*, and *Yersinia* (Table 1).

PCR results of samples that had undergone phenol-chloroform extraction showed that there was uneven amplification of the different DNA sequences and some sequences occasionally were not amplified to detectable levels. In initial experiments, 25 g of bovine feces was used for the enrichment cultures; PCR results showed no bands and only a smear on the gel even after organic extraction (data not shown). When 5 g of feces was used, results were more favorable. In multiplex PCR, uneven amplification of target sequences could be due to insufficient concentration of deoxynucleoside triphosphates or of the DNA polymerase. Therefore, a variety of reaction conditions were tested in order to optimize the PCR. The concentration of *Taq* DNA polymerase was doubled from 1.25 to 2.5 U per reaction increasing the probability that each primer/template hybrid will be extended by the enzyme. The $MgCl_2$ concentration was increased from 1.5 to 3 mM per reaction and the dNTP concentration was increased from 200 to 400 μm per reaction. Since the plasmid and *stx* sequences did not appear to be amplified as efficiently as the other sequences, the concentration of MFS1F, MFS1R, MK1 and MK2 primers was increased from 25 to 50 μM in each reaction while the concentration of the other primers was kept at 25 μM.

Results obtained with samples that had undergone DNA extraction using the High Pure PCR Template Preparation Kit were superior to those obtained following the organic extraction procedure. Results obtained using samples subjected to organic extraction were often not reproducible and frequently the intensities of the PCR products were unequal (data not shown). PCR products resulting from amplification of the plasmid sequence (166 bp), stx_2 (225 bp), *eaeA* (397 bp), and *invA* (796 bp) genes were visible in all the samples of enrichment cultures of artificially-inoculated apple cider, carcass wash water, and ground beef that had undergone DNA extraction using the commercially available DNA extraction kit (Figure 2). The limit of detection was $\leq$ 1 CFU of both organisms g^{-1} of ground beef or ml^{-1} of apple cider (Figure 2). The detection limit of the carcass wash water samples was even lower at $<$ 0.1 CFU ml^{-1}. In these experiments the concentration of MK1 and MK2 used in each reaction was 0.25 μM and the amplification product derived from the *stx* sequence was weaker than the other bands. Subsequently, we increased the concentration of those primers to 50 μM per reaction.

The ground beef and bovine fecal samples underwent enrichment in both mEC + n and BPW + n. In samples processed with the DNA extraction kit and samples in which bacteria were concentrated by IMS, *E. coli* O157:H7 and

Table 2 Oligonucleotides used as primers in multiplex PCR

Oligonucleotide primer	Sequence (5′ to 3′)	Target gene	Expected size of PCR product (bp)
AE22	ATTACCATCCACACAGACGGT	*eaeA*	397
AE20-2	ACAGCGTGGTTGGATCAACCT		
MK1	TTTACGATAGACTTCTCGAC	stx_1/stx_2	228/225
MK2	CACATATAAATTATTTCGCTC		
MFS1F	ACGATGTGGTTTATTCTGGA	Plasmid	166
MFS1R	CTTCACGTCACCATACATAT		
INVAF	CGGTGGTTTTAAGCGTACTCTT	*invA*	796
INVAR	CGAATATGCTCCACAAGGTTA		

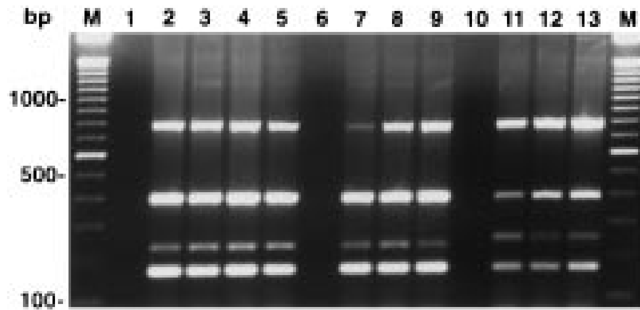

Figure 2 Ethidium bromide-stained agarose gel showing PCR products obtained following multiplex PCR amplification of *E. coli* O157:H7 and *S. typhimurium* DNA recovered from artificially-inoculated apple cider, beef carcass wash water and ground beef samples. Lanes M, DNA size markers (100 bp DNA ladder, Gibco/BRL); lanes 1–5 show amplification products from an uninoculated apple cider sample (lane 1), and from apple cider (25 ml) inoculated with approximately 3 CFU of *E. coli* O157:H7 and 25 CFU of *S. typhimurium* (lane 2), 6 CFU of *E. coli* and 100 CFU of *S. typhimurium* (lane 3), 15 CFU of *E. coli* and 250 CFU of *S. typhimurium* (lane 4) and 250 CFU of both *E. coli* and *S. typhimurium* (lane 5); lanes 6, 7, 8 and 9 show amplification products from uninoculated beef carcass wash water, wash water (300 ml) inoculated with 3 CFU of *E. coli* and 25 CFU of *S. typhimurium*, inoculated with 6 CFU of *E. coli* and 100 CFU of *S. typhimurium* and with 15 and 250 CFU of both *E. coli* and *S. typhimurium*, respectively; lanes 10, 11, 12 and 13 show amplification products from an uninoculated ground beef sample (25 g) and inoculated with 3 CFU of *E. coli* and 25 CFU of *S. typhimurium*, 6 CFU of *E. coli* and 100 CFU of *S. typhimurium* and with 15 CFU of *E. coli* and 250 CFU of *S. typhimurium*, respectively.

S. typhimurium were detectable in both mEC + n and BPW + n enrichments at a level of $\leq$ 1 CFU g^{-1} of bovine feces (Figures 3 and 4). All four bands of the expected sizes were clearly visible on the gels. Nonspecific bands were not visible except occasionally one weak band appeared in uninoculated control samples processed using the DNA extraction kit. However, the sizes of the nonspecific bands were different from the sizes of the expected *E. coli* O157:H7 and *S. typhimurium* amplification products. Results using ground beef enrichments were similar.

Plating and PCR results indicated that both *E. coli* O157:H7 and *Salmonella* grew well in both mEC + n and in BPW + n and typical *E. coli* O157:H7 and *S. typhimurium* colonies formed on the agars (data not shown). On XLT4, *Salmonella* appear as black colonies, on BG Sulfa agar *Salmonella* colonies appeared pink and it took longer for colonies to form on BG Sulfa than XLT4 agar. *E. coli* O157:H7 appear light pink on CT-SMAC and black on Rainbow agar. CT-SMAC was considerably more selective than Rainbow agar. Several of the colonies were selected from the various agars and confirmed as either *E. coli* O157:H7 or *S. typhimurium* by PCR using primers specific for each organism.

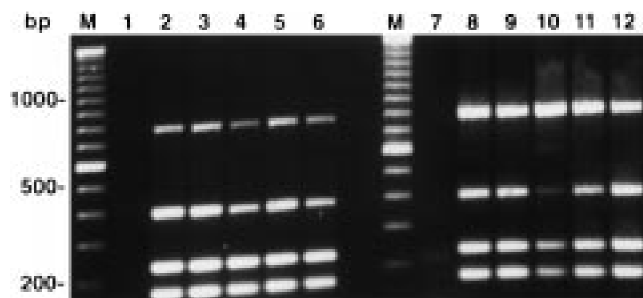

Figure 3 Ethidium bromide-stained agarose gel showing PCR products obtained following multiplex PCR amplification of *E. coli* O157:H7 and *S. typhimurium* DNA recovered from artificially-inoculated bovine feces using the High Pure PCR Preparation Kit. Lanes M, DNA size markers; lanes 1–6 show products of BPW + n enrichments and lanes 7–12 show PCR products of mEC + n enrichments. Lanes 1 and 7, uninoculated controls; lanes 2 and 8, samples inoculated with *ca* 1 CFU g^{-1} of feces each of *E. coli* O157:H7 and *S. typhimurium*; lanes 3 and 9, with *ca* 5 CFU g^{-1}; lanes 4 and 10, *ca* 10 CFU g^{-1}; and lanes 6 and 12, *ca* 100 CFU g^{-1}.

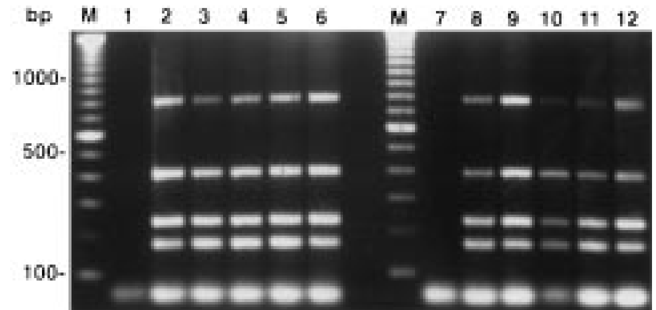

Figure 4 Ethidium bromide-stained agarose gel showing PCR products obtained following recovery of the target bacteria by IMS and multiplex PCR amplification of *E. coli* O157:H7 and *S. typhimurium* DNA from artificially-inoculated bovine feces. Lanes M, DNA size markers; lanes 1–6 show products of BPW + n enrichments and lanes 7–12 show PCR products of mEC + n enrichments. Lanes 1 and 7, uninoculated controls; lanes 2 and 8, samples inoculated with *ca* 1 CFU g^{-1} of feces each of *E. coli* O157:H7 and *S. typhimurium*; lanes 3 and 9, with *ca* 5 CFU g^{-1}; lanes 4 and 10, 10 CFU g^{-1}; and lanes 6 and 12, 100 CFU g^{-1}.

Discussion

We have previously reported on the use of a multiplex PCR for direct detection of *E. coli* O157:H7 in enrichment cultures of ground beef and raw milk [8] and for identification of suspect colonies on selective/differential medium [7]. The primer sets for amplification of plasmid and *stx* sequences used in the present study were the same as those described previously [7]. For amplification of the *eaeA* gene sequence, primer AE20 was also the same as that described previously, except the first T at the 5′ end was replaced with an A (primer now designated AE20-2). In a multiplex PCR, more efficient amplification is achieved if the sizes of the amplified DNA products are close to one another, therefore, a different forward primer (AE22) was used in order to obtain an *eaeA* gene amplification product smaller than 1089 bp. Primer set INVAF and INVAR for amplification of a *Salmonella invA* gene sequence was designed using the OLIGO program (NBI/Genovus, Plymouth, MN, USA).

There are over 2300 serovars of *Salmonella* and approximately 150 have been associated with human disease; however, the remaining serovars are also potential human pathogens. Most cases of food-borne disease caused by *Salmonella* have been associated with *S. typhimurium* or *S. enteritidis*. The *Salmonella invA* gene has been characterized and found to be highly conserved in the genus *Salmonella* [9]. Hybridization and PCR assays for specific detection of the organism in foods based on detection of *invA* sequences have been reported [2,3].

Yu and Bruno [20] described an immunomagnetic electrochemiluminescent assay to detect *E. coli* O157 and *Salmonella* in foods and water samples. The assay was rapid and rather sensitive; however, it was not entirely specific since some degree of cross-reactivity of the antibodies with other organisms was observed. Furthermore, the antibodies used for detection of *E. coli* were not H7-specific, therefore, the presence in the sample of *E. coli* O157 of other H groups would also produce a positive result. In contrast, genetic-based detection assays can be designed to be extremely specific if unique nucleic acid sequences are targeted.

While PCR is a very attractive technique for detection and identification of bacteria, there are problems associated with testing complex samples that need to be overcome before successful detection can be achieved. The usefulness of PCR for detection of microorganisms in foods and animal fecal specimens is limited by the presence of substances that inhibit the DNA polymerase, bind magnesium or denature the DNA. The presence of PCR inhibitors in these types of samples significantly decreases sensitivity of the assay. For example, complex polysaccharides present in fecal specimens and high amounts of fat and proteins in food samples can notably affect PCR performance [12,16].

IMS is an elegant method for concentration of target organisms and separation of the bacteria from PCR-inhibitory components present in enrichment cultures. The technique has been used to capture *E. coli* O157:H7 from enrichments of dairy products prior to PCR amplification [10]. In the present study, PCR results obtained with IMS and DNA extraction treatments of ground beef and bovine fecal sample enrichments were similar. *E. coli* O157:H7 and *Salmonella* were both detected at levels of ≤ 1 CFU g^{-1}.

Heuvelink *et al* [11] compared the efficacy of various types of media for selective enrichment and isolation of *E. coli* O157 and found that mEC + n for enrichment and CT-SMAC for isolation of the organism were optimal. In the present study, mEC + n and BPW + n were compared to determine if both media allowed for growth of both *E. coli* O157:H7 and *S. typhimurium* to detectable levels. Plating on the selective agars and PCR results showed that recovery and detection of the organisms from bovine fecal and ground beef enrichments using the two media can be achieved. An advantage of using BPW for enrichment is that it contains no selective agents such as bile salts; therefore, it is more likely that any injured cells will be recovered. Plating onto the selective agars allowed for isolation of *E. coli* O157:H7 and *S. typhimurium* from ground beef and bovine fecal enrichment cultures. An advantage of performing PCR for detection of bacteria in foods is that there is usually no interference from background microflora as may occur in testing procedures which involve plating unless highly selective agars are used. However, highly selective plating media may also inhibit growth of target organisms to some degree. Plating of enrichment cultures may be necessary if testing methods require isolated colonies for further investigation.

The multiplex PCR assay described in this study can be used to detect *E. coli* O157:H7 and *Salmonella* directly from enrichment cultures; therefore, the time required for testing for these organisms can be shortened considerably. Detection of the target organisms is possible within 30 h from the time of receipt of samples. Use of this assay can potentially permit the food industry and regulatory agencies to more readily evaluate foods and other types of samples for the presence of *E. coli* O157:H7 and *Salmonella* thus enhancing the safety of the food supply.

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