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A Multiplex Polymerase Chain Reaction Assay for Rapid Detection and Identification of *Escherichia coli* O157:H7 in Foods and Bovine Feces†

PINA M. FRATAMICO,[1]* LORI K. BAGI,[1] AND TIZIANA PEPE[2]

[1]*Agricultural Research Service, Eastern Regional Research Center, U.S. Department of Agriculture, 600 E. Mermaid Lane, Wyndmoor, Pennsylvania 19038, USA; and* [2]*University of Naples Federico II, Department of Pathology, Prophylaxis, and Food Inspection, Via F. Delpino 1, 80137 Naples, Italy*

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ABSTRACT

A multiplex polymerase chain reaction (PCR) assay was designed to simplify detection of *Escherichia coli* O157:H7 and to identify the H serogroup and the type of Shiga toxin produced by this bacterium. Primers for a plasmid-encoded hemolysin gene (hly_{933}), and chromosomal flagella ($fliC_{h7}$; flagellar structural gene of H7 serogroup), Shiga toxins (stx_1, stx_2), and attaching and effacing (*eaeA*) genes were used in a multiplex PCR for coamplification of the corresponding DNA sequences from enterohemorrhagic *E. coli* (EHEC) O157:H7. Enrichment cultures of ground beef, blue cheese, mussels, alfalfa sprouts, and bovine feces, artificially inoculated with various levels of *E. coli* O157:H7 strain 933, were subjected to a simple DNA extraction step prior to the PCR, and the resulting amplification products were analyzed by agarose gel electrophoresis. Sensitivity of the assay was ≤1 CFU/g of food or bovine feces (initial inoculum level), and results could be obtained within 24 h. Similar detection levels were obtained with ground beef samples that underwent enrichment culturing immediately after inoculation and samples that were frozen or refrigerated prior to enrichment. The multiplex PCR facilitates detection of *E. coli* O157:H7 and can reduce the time required for confirmation of isolates by up to 3 to 4 days.

Infection with *Escherichia coli* O157:H7 remains a major public health concern in the United States and worldwide. The organism causes severe diarrheal illness that can potentially lead to life-threatening complications such as hemolytic uremic syndrome *(6).* Although *E. coli* O157:H7 is principally associated with foods of bovine origin, in recent years a wide variety of foods such as goat's milk, lettuce, apple cider, and alfalfa sprouts have also served as vehicles of transmission *(1–3, 6).* Pathogenic features of *E. coli* O157:H7 include the production of Shiga toxin 1 (stx_1) and/or Shiga toxin 2 (stx_2), the possession of a locus of enterocyte effacement containing an *eaeA* gene that encodes a protein called intimin *(4),* and the possession of an ~60-MDa plasmid that encodes a hemolysin *(7, 15).*

We reported in an earlier study *(7)* that sequencing of a portion of a 3.4-kb fragment of the 60-MDa plasmid of *E. coli* O157:H7 strain 933 cloned into pCVD419 *(14)* revealed 63.5% homology between the sequenced region and a chromosomal *E. coli hlyA* gene. We refer to this *E. coli* O157:H7 gene as hly_{933}. It is believed that hly_{933} may be indistinguishable from enterohemorrhagic *E. coli* (EHEC)-*hlyA* described by Schmidt et al. *(22),* because they found that a probe prepared from pCVD 419 harbored most of the EHEC-*hlyA* and the 5′ region of EHEC-*hlyB.*

In *E. coli,* the *fliC* gene encodes flagellin proteins of different antigenic character. In conventional detection methodologies, determination of the H7 antigen and of the Shiga toxin type is usually performed on presumptive positive colonies isolated from selective/differential media *(18).* Serological testing using flagellar antigen-specific antisera is performed to confirm that an isolate is of the H7 serogroup. With some isolates, confirmation of H7 serology may be delayed, because multiple passages may be required before the flagellar antigen is detectable. The type of toxin produced by an isolate is determined by a relatively cumbersome and time-consuming tissue culture technique and/or by DNA hybridization or enzyme-linked immunosorbent assays *(20).* Therefore, it may require several days for definitive identification of *E. coli* O157:H7.

Numerous reports on applications of polymerase chain reaction (PCR)-based assays for detection of pathogens in foods have appeared *(10, 19).* The usefulness of the PCR for detection of microorganisms in food and other complex samples, however, is limited by the presence of substances that inhibit DNA polymerase, bind magnesium, and/or denature DNA. Sensitivity is decreased dramatically if the sample contains inhibitors; therefore, extensive sample preparation and DNA extraction steps are usually required prior to the PCR *(13).* In the present study, a very simple and rapid DNA extraction procedure, using the commercially available PrepMan Sample Preparation Reagent, was performed on enrichment cultures of various foods and on bovine feces inoculated with *E. coli* O157:H7. To our knowledge, this is the first peer-reviewed report on the use of this reagent for PCR template preparation from enrich-

* Author for correspondence. Tel: 215-233-6525; Fax: 215-233-6581; E-mail: pfratamico@arserrc.gov.

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ments of food or bovine feces. The samples were then subjected to multiplex PCR using primers targeting the *eaeA*, stx_1, stx_2, $fliC_{h7}$, and hly_{933} genes of *E. coli* O157:H7. This multiplex PCR permits detection of *E. coli* O157:H7 in various types of complex samples, identification of the H7 serogroup, and identification of the types of Shiga toxin produced in a single assay, resulting in savings in cost and time.

MATERIALS AND METHODS

Bacteria. *E. coli* O157:H7 strain 933 (ground beef isolate; possesses both stx_1 and stx_2) was obtained from the U.S. Department of Agriculture Food Safety and Inspection Service (Athens, Ga.). *E. coli* strains used to test the specificity of the $fliC_{h7}$ primers by PCR were obtained from our laboratory culture collection and included strains of the following serogroups: NM (nonmotile), H^-, H1, H2, H5, H6, H7 (including O157:H7 and non-O157 strains), H8, H11, H12, H16, H19, H21, H25, H27, H41, and H45. The cultures were maintained on tryptic soy agar (Difco Laboratories, Detroit, Mich.) slants at 4°C. The cultures were inoculated into 50 ml of brain heart infusion (Difco) broth and grown at 37°C for 18 h at 150 rpm prior to each experiment.

Enrichment culturing of foods and bovine feces. Tenfold serial dilutions of 18-h cultures of *E. coli* O157:H7 strain 933 were made in 0.1% peptone, resulting in bacterial concentrations of approximately 1, 10, 100, 1,000, and 10,000 CFU/ml. To determine the actual numbers of bacteria, each dilution was plated onto brain heart infusion agar plates and colonies were enumerated after incubation at 37°C for 18 h.

Alfalfa sprouts, blue cheese, mussels, and ground beef were purchased from local supermarkets and generally used on the day of purchase. Bovine feces were obtained from a local agricultural college and stored at −20°C until used *(8)*. The mussels, including the liquid present, were removed from the shells, placed in filter-Stomacher bags (Bel-Art Products, Pequannock, N.J.), and pummeled for 2 min in a Stomacher Lab-Blender (model 400; Tekmar Co., Cincinnati, Ohio). The bovine feces, alfalfa sprouts, blue cheese, and ground beef were not pummeled using a Stomacher.

The medium used for enrichment culturing was modified *E. coli* broth containing novobiocin at 0.02 mg/ml (mEC+n) *(18)*. To 90 ml of mEC+n in a 500-ml–volume Erlenmeyer flask, 2.5 ml of the various dilutions of *E. coli* O157:H7 and 10 g of food were added. Instead of 10 g, 2 g of bovine feces were added to 98 ml of mEC+n along with 2.5 ml of the bacterial dilutions. The samples were incubated at 37°C for 24 h at 150 rpm. Dilutions in 0.1% peptone were made of the alfalfa sprout and ground beef 24-h enrichments and portions thereof were plated onto Rainbow Agar O157 (Biolog, Inc., Hayward, Calif.). As instructed by the manufacturer, the agar was prepared with 0.8 mg/liter of tellurite and 10 mg/liter of novobiocin to increase selectivity. *E. coli* O157:H7 appear as black or dark gray colonies on this agar after growth for 20 to 24 h or longer at 35 to 37°C.

Samples frozen or refrigerated prior to enrichment. Duplicate 10-g samples of ground beef were inoculated with 2.5 ml of *E. coli* O157:H7 suspensions at concentrations of 1, 10, and 100 CFU/ml and added to 90 ml of mEC+n broth. Three sets of samples were inoculated. One set was sampled immediately after inoculation and then incubated at 37°C for enrichment. The second and third sets were stored at 4°C or −20°C, respectively, for 48 h prior to enrichment. Samples held at −20°C were thawed at room temperature prior to addition to mEC+n broth. One-milliliter portions were removed after 6, 8, 12, and 24 h of enrichment culturing and subjected to DNA extraction and the PCR as described below.

DNA extraction and immunomagnetic separation. Extraction of DNA from all of the samples that underwent enrichment was performed using the PrepMan Sample Preparation Reagent (Perkin Elmer-Applied Biosystems Division, Foster City, Calif.) according to the manufacturer's instructions. Briefly, 1 ml of each enrichment culture was separately transferred to microcentrifuge tubes. The cells were harvested by centrifugation for 2 min at 16,000 × *g*. The supernatant was removed, and 200 μl of PrepMan reagent was added to the pellet. After mixing well to resuspend the pellet, the samples were heated in a boiling water bath for 10 min and cooled to room temperature. The tubes were again subjected to centrifugation for 2 min at 16,000 × *g*, and a 5-μl aliquot of the supernatant was used for the PCR.

E. coli O157:H7 was recovered from the enrichment cultures of the inoculated alfalfa sprouts by immunomagnetic separation (IMS). One milliliter of the samples was mixed with 20 μl of Dynabeads anti-*E. coli* O157 (Dynal A.S., Oslo, Norway) in 1.5-ml microcentrifuge tubes, and the samples were processed as described previously *(8)*. The tubes were heated to 99°C for 10 min in a Perkin-Elmer GeneAmp PCR System 9600 thermal cycler (Perkin-Elmer Applied Biosystems) to lyse the bacteria.

Specificity of FLICH7 primers. The FLICH7-F and FLICH7-R primers *(9)* were obtained from GIBCO BRL Life Technologies, Inc. (Gaithersburg, Md.) and tested for specificity using several *E. coli* O157:H7 strains and strains of other H serogroups (listed above). Freshly grown bacterial colonies from brain heart infusion agar plates were suspended in 40 μl of sterile H_2O and heated at 99°C for 10 min in a thermal cycler; 5 μl of the resulting lysates were used for the PCR. The PCR conditions for amplification of the *E. coli* $fliC_{h7}$ gene were as described below (Multiplex PCR protocol), except only the FLICH7 primer set was used, and the $MgCl_2$ concentration was decreased to 1.5 mM. Amplification products were visualized following electrophoresis through 1.5% agarose gels stained with ethidium bromide.

Multiplex PCR protocol. The oligonucleotide primers used in the multiplex PCR and the sizes of the expected PCR products are listed in Table 1. Primer set SLT-I and SLT-II *(16)* was obtained from GIBCO BRL Life Technologies, Inc. Primers MFS1-F, MFS1-R, AE20-2, and AE22 *(8)* were obtained from Synthetic Genetics (San Diego, Calif.). The PCR Reagent System Kit (GIBCO) was employed to prepare the PCR mixture that 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), 2.5 U *Taq* DNA polymerase, and 0.50 μM of all primers except AE22 and AE20-2 that were used at 0.25 μM. A 5-μl portion of extracted DNA and of supernatant recovered from the bead mixture following IMS and lysis were each added to 200-μl-volume tubes containing 45 μl of the PCR reaction mixture. The PCR reaction samples were heated at 94°C for 2 min and then subjected to 35 cycles of denaturation at 94°C for 20 s, annealing at 57°C for 1 min, and extension at 72°C for 1 min, with a final extension for 10 min at 72°C. Products of the multiplex PCR were visualized following electrophoresis through 1.5% agarose gels stained with ethidium bromide.

RESULTS

The sizes of the PCR products obtained following multiplex PCR of enrichment cultures of samples seeded with *E. coli* O157:H7 strain 933 were 625, 210, 484, 397, and 166 bp, as expected from the $fliC_{h7}$, stx_1, stx_2, *eaeA*, and

TABLE 1. *Oligonucleotide primers used in the multiplex PCR*

Oligonucleotide primers	Sequence (5′ to 3′)	Target gene	Expected size of the PCR product (bp)	Reference
FLICH7-F	GCGCTGTCGAGTTCTATCGAGC	*fliC*$_{h7}$	625	*9*
FLICH7-R	CAACGGTGACTTTATCGCCATTCC			
SLTI-F	TGTAACTGGAAAGGTGGAGTATACA	*stx*$_1$	210	*16*
SLTI-R	GCTATTCTGAGTCAACGAAAAATAAC			
SLTII-F	GTTTTTCTTCGGTATCCTATTCC	*stx*$_2$	484	*16*
SLTII-R	GATGCATCTCTGGTCATTGTATTAC			
AE22	ATTACCATCCACACAGACGGT	*eaeA*	397	*8*
AE20-2	ACAGCGTGGTTGGATCAACCT			
MFS1-F	ACGATGTGGTTTATTCTGGA	*hly*$_{933}$ (plasmidborne)	166	*7*
MFS1-R	CTTCACGTCACCATACATAT			

hly$_{933}$ sequences, respectively (Figs. 1 and 2). A number of *E. coli* strains belonging to various H serogroups were tested with the FLICH7 primers to determine specificity. The PCR amplification yielded only a 625-bp product with all *E. coli* O157:H7 strains and the single *E. coli* O157:NM (i.e., nonmotile) strain tested (data not shown). The nonmotile O157 strain was sorbitol negative and positive for *stx*, *eaeA*, and the plasmid-encoded hemolysin sequence when tested previously by multiplex PCR *(7)*.

Detection of *E. coli* O157:H7 in seeded alfalfa sprouts. To remove substances that could inhibit the PCR, the enrichment cultures of the seeded alfalfa sprouts were processed by IMS to recover and concentrate target bacteria and by the PrepMan procedure to extract the DNA. The five amplicons (products of *fliC*$_{h7}$, *stx*$_1$, *stx*$_2$, *eaeA*, and *hly*$_{933}$ sequences) were visible on agarose gels following PCR amplification of alfalfa sprout samples initially inoculated with levels of *E. coli* O157:H7 as low as 1 CFU/g (Fig. 1). Similar results were observed with samples processed by IMS or using PrepMan. After a 24-h enrichment at 37°C, the number of black colonies counted on Rainbow Agar O157 corresponded to 4.8 $\log_{10}$ CFU/ml of enrichment in samples inoculated with 1 CFU/g. In samples inoculated with 10 CFU/g the number of black colonies counted corresponded to 7.4 $\log_{10}$ CFU/ml, and in samples inoculated with 100 to 10,000 CFU/g, the number of black colonies corresponded to 8.3 to 8.8 $\log_{10}$ CFU/ml of enrichment culture.

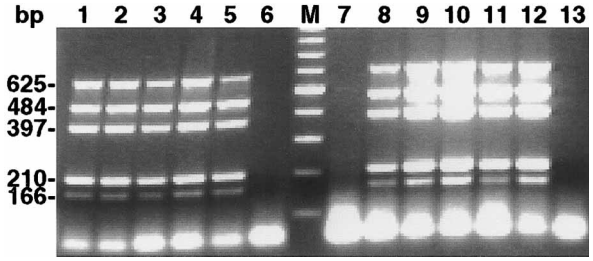

FIGURE 1. *Ethidium bromide-stained agarose gel showing amplicons obtained following PCR amplification of inoculated alfalfa sprout samples. Lanes 1 through 6 show results of enrichment samples that were subjected to IMS prior to the PCR. Lanes 7 through 12 show results of samples extracted using the PrepMan reagent prior to the PCR. Lanes marked 1 through 5 and 8 through 12 show results of samples that were inoculated with approximately 1 CFU/g (lanes 1 and 8), 10 CFU/g (lanes 2 and 9), 100 CFU/g (lanes 3 and 10), 1,000 CFU/g (lanes 4 and 11), and 10,000 CFU/g (lanes 5 and 12). All samples were subjected to a 24-h enrichment. Lanes 6 and 7 show results of uninoculated sprouts and lane 13 is the negative control (5 μl of sterile water instead of sample); no bands were visible. Lane M, 100-bp ladder DNA size markers.*

Effect of enrichment time and cold stress on detection of *E. coli* O157:H7 in seeded ground beef samples. To determine the shortest enrichment time needed to allow detection of low levels of the organism by PCR, and to determine if bacteria exposed to cold stress were detectable using the enrichment protocol, ground beef samples were subjected to enrichment immediately after inoculation or after inoculation and storage at either 4°C or −20°C for 48 h before multiplex PCR analysis. Generally, the PCR results of samples subjected to enrichment immediately after inoculation or after inoculation followed by cold storage were similar. As shown in Figure 2, following a 6-h enrichment, all five PCR products of *E. coli* O157:H7 strain 933 were detected in samples inoculated with 100 CFU/g (lane 3) but not in samples that contained 10 or 1 CFU/g (lanes 2 and 1, respectively). Following an 8-h enrichment, all five PCR products of strain 933 were visible in the sample inoculated with 10 CFU/g (lane 5), whereas following a 12-h enrichment, the five PCR products were visible in samples inoculated with approximately 1 CFU/g (lane 8).

With samples that were refrigerated for 2 days prior to enrichment, all five PCR products were visible following a 6-h enrichment in samples inoculated with 10 CFU/g (Fig. 2, lane 13) and visible following an 8-h enrichment in samples inoculated with 1 CFU/g (lane 15). Results with samples frozen prior to enrichment (lanes 21–30) were similar to those observed with samples not subjected to cold storage prior to enrichment. The five PCR products were visible after a 6-h enrichment in samples inoculated with 100 CFU/g (lane 24). Therefore, bacteria subjected to short periods of cold stress are detectable by PCR after enrichment for the same length of time required to detect bacteria not

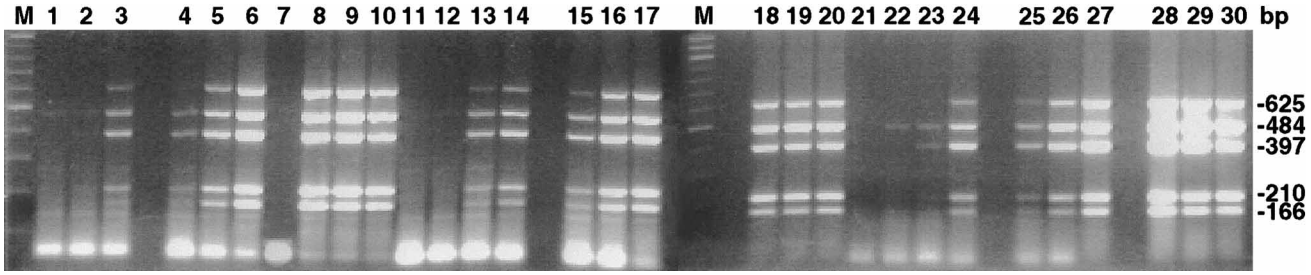

FIGURE 2. *Ethidium bromide-stained agarose gel showing amplification products obtained following PCR amplification of inoculated ground beef samples. Lanes 1 through 10, samples that underwent enrichment immediately after inoculation at levels of 1 CFU/g (lanes 1, 4, 8, 12, 15, 18, 22, 25, 28), 10 CFU/g (2, 5, 9, 13, 16, 19, 23, 26, 29), and 100 CFU/g (3, 6, 10, 14, 17, 20, 24, 27, 30); lanes 11 through 20 and lanes 21 through 30, samples that underwent enrichment culturing after inoculation and storage at 4°C and −20°C, respectively. Lanes 1 through 3, 12 through 14, and 22 through 24, samples that were tested after a 6-h enrichment; lanes 4 through 6, 15 through 17, and 25 through 27, after an 8-h enrichment; and lanes 8 through 10, 18 through 20, and 28 through 30, after a 12-h enrichment. Lanes 7, 11, and 21, uninoculated samples tested after a 12-h enrichment. Lanes M, 100-bp ladder DNA size markers.*

subjected to cold stress. After 24 h of enrichment, samples stored for 2 days at 4°C and −20°C were plated onto Rainbow Agar O157. The number of black colonies counted corresponded to 5.3 and 7.0 $\log_{10}$ CFU/ml of enrichment culture in samples stored at 4°C for 2 days and initially inoculated with 1 and 100 CFU/g, respectively, and 5.6 and 6.9 $\log_{10}$ CFU/ml of enrichment in samples stored at −20°C and inoculated with 1 and 100 CFU/g, respectively.

Detection of *E. coli* O157:H7 in mussels, blue cheese, and bovine feces. Other samples including mussels, blue cheese, and bovine feces were inoculated with *E. coli* O157:H7 strain 933 and subjected to enrichment, extracted using PrepMan, and analyzed by the multiplex PCR. *E. coli* O157:H7 was detectable at ≤1 CFU/g of mussels, blue cheese, and bovine feces after a 24-h enrichment at 37°C and 150 rpm (data not shown). Samples of inoculated mussels, blue cheese, alfalfa sprouts, and bovine feces were not subjected to cold storage prior to analysis by multiplex PCR; however, results would not be expected to differ considerably from those obtained with the ground beef samples that were subjected to cold storage. Also, enrichments of mussels, blue cheese, ground beef, and bovine feces were not subjected to IMS to remove PCR-inhibiting substances; however, again results would not be expected to differ considerably from those obtained with the alfalfa sprout samples. A previous study showed that IMS was effective for removal of PCR-inhibiting substances from enrichments of bovine feces and ground beef *(8).*

DISCUSSION

The multiplex PCR assay described in this report entails simultaneous amplification of five different *E. coli* O157:H7 DNA sequences followed by detection of the amplified products by agarose gel electrophoresis. The amplified sequences are portions of the chromosomal $fliC_{h7}$, stx_1, stx_2, and *eaeA* genes and of a portion of the hemolysin gene located on the resident 60-MDa plasmid. Moreover, the resulting five PCR amplification products generate a fingerprint of this organism on agarose gels that may be useful for identification. Targeting any of the five sequences individually would not allow identification because a number of *E. coli* serotypes produce Shiga toxins *(11)* and/or may possess the 60-MDa plasmid *(7).* A PCR product from the *eaeA* gene would be obtained from *E. coli* O55:H7 strains *(7),* and a PCR product using the FLICH7 primers would be obtained with non-O157 *E. coli* belonging to the H7 serogroup.

Using conventional *E. coli* O157:H7 protocols *(18),* confirmatory testing requires identification of the H7 flagellar antigen, because isolates positive for the O157 antigen by latex agglutination may possess H antigens other than H7. Some O157 strains have required numerous passages in motility medium or motility enhancement procedures to obtain expression of flagella, thus delaying confirmation of isolates. The FLICH7 primers were designed by Gannon et al. *(9)* from the *fliC* sequence of *E. coli* O157:NM strain E32511. Using these primers in the present study, a Shiga-toxin-producing *E. coli* strain identified as O157:NM was found to harbor the $fliC_{h7}$ gene.

In addition to serological and biochemical analyses, Shiga toxin neutralization tissue culture assays may be performed to determine production of Shiga toxin 1 and/or Shiga toxin 2 by *E. coli* O157:H7 isolates. With the multiplex PCR, if the isolate possesses either the stx_1 or stx_2 genes, a product of 210 or 484 bp is obtained, respectively, and both products are obtained if the strain harbors both toxin genes. Furthermore, non-O157:H7 *E. coli* strains that produce Shiga toxins can be detected in food or other types of samples by the multiplex PCR.

The multiplex PCR was used for detection of *E. coli* O157:H7 in blue cheese, mussels, alfalfa sprouts, ground beef, and bovine feces after a simple DNA extraction step using the PrepMan reagent. The organism was detected after a 24-h enrichment in samples inoculated with ≤1 CFU/g. Polysaccharides and polyphenolic compounds found in most higher plants can inhibit the PCR by forming complexes with nucleic acids *(12).* Thus, two procedures were employed to remove inhibiting substances from enrichment cultures of alfalfa sprouts: IMS using beads coated with antibodies against *E. coli* O157:H7 and DNA extraction using PrepMan. For removal of PCR-inhibiting substances, both procedures were equally effective; five PCR products were visible on gels with samples that were inoculated with 1 CFU/g (Fig. 1). With IMS, however, saturation of the

antibody binding sites occurs; band intensity was equal with all samples despite plating results confirming differences in the number of *E. coli* O157:H7 present that corresponded with the different initial inoculum levels. In contrast, variations in band intensities were observed in otherwise similar samples treated with PrepMan; samples inoculated with 1 CFU/g displayed less intense bands.

Animal feces also contain numerous materials, including complex polysaccharides, bilirubin, and bile salts that are inhibitors of PCR *(17, 25).* A previous study showed that no amplification products were observed when enrichments of bovine feces were used directly for the PCR, and organic extraction of enrichments also did not yield suitable results *(8).* Components in cheese such as fat and proteinases can act as strong inhibitors of the PCR *(21, 24).* A single template preparation procedure may not be suitable for removal of inhibiting components from a wide variety of samples. In this study, PrepMan was a relatively rapid and simple extraction method that worked reliably on different food types and on animal feces. Lipophilic and hydrophilic PCR-interfering sample components are sequestered by PrepMan and other debris are removed by centrifugation. The procedure yielded templates suitable for PCR amplification.

Doyle and Schoeni *(5)* found that after 9 months of storage at −20°C, there was only a small decrease in the number of *E. coli* O157:H7/g of inoculated ground beef (6,700 CFU at day 0 and 6,200 CFU after 9 months of storage at −20°C). Surviving bacteria were determined by blending the frozen meat and plating dilutions onto trypticase soy agar overlaid with MacConkey agar. Thus, it is not unexpected that in the current study, the ability to detect *E. coli* O157:H7 by the multiplex PCR in inoculated ground beef was not affected by cold stress (storage at 4 and −20°C for 2 days). After an 8-h enrichment, *E. coli* O157:H7 was detected (five bands visible on ethidium bromide-stained agarose gels) in samples inoculated with 10 CFU/g and enriched immediately after inoculation or enriched after 2 days at 4°C or −20°C. However, Uyttendaele et al. *(23)* found that prolonged storage of ground beef inoculated with *E. coli* O26:H11 or O157:H$^-$ influenced the enrichment time required for positive detection by PCR. Samples in which bacteria were subjected to cold stress at 4°C for 14 days required about 3.5 h longer enrichment than samples that underwent enrichment immediately after inoculation. The investigators used non-O157:H7 *E. coli* serotypes for ground beef inoculation and subjected the meat to periods of cold stress longer than 2 days, which may account for the differences in results compared with the present study.

The multiplex PCR allows for rapid detection, and possibly identification, of *E. coli* O157:H7 in foods and other types of samples present at levels of ≤1 CFU/g prior to enrichment. It markedly reduces the time required for confirmation of isolates because lengthy biochemical, serological, and toxin testing could potentially be eliminated. Studies utilizing the multiplex PCR assay for detection of *E. coli* O157:H7 in other types of foods, both naturally and artificially contaminated, are ongoing.

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