APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 1992, p. 3809–3815
0099-2240/92/123809-07$02.00/0

Vol. 58, No. 12

Rapid and Sensitive Method for Detection of Shiga-Like Toxin-Producing *Escherichia coli* in Ground Beef Using the Polymerase Chain Reaction

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A rapid and sensitive method for detection of Shiga-like toxin (SLT)-producing *Escherichia coli* (SLT-EC) with the polymerase chain reaction (PCR) is described. Two pairs of oligonucleotide primers homologous to SLTI and SLTII genes, respectively, were used in multiplex PCR assays. The first pair generated a ca. 600-bp PCR product with DNA from all SLTI-producing *E. coli* tested but not from *E. coli* strains that produce SLTII or variants of SLTII. The second pair generated a ca. 800-bp PCR product with DNA from *E. coli* strains that produce SLTII or variants of SLTII but not from SLTI-producing *E. coli*. When used in combination, the SLTI and SLTII oligonucleotide primers amplified DNA from all of the SLT-EC tested. No PCR products were obtained with SLT primers with DNA from 28 *E. coli* strains that do not produce SLT or 44 strains of 28 other bacterial species. When ground beef samples were inoculated with SLT-EC strains 319 (O157:H7; SLTI and SLTII), H30 (O26:H11; SLTI), and B2F1/3 (O91:H21; SLTII variants VT2ha and VT2hb) and cultured in modified Trypticase soy broth for 6 h at 42°C, an initial sample inoculum of as few as 1 CFU of these SLT-EC strains per g could be detected in PCR assays with DNA extracted from the broth cultures.

Shiga-like toxin (SLT)-producing *Escherichia coli* (SLT-EC), also known as Verotoxin-producing *E. coli*, are associated with infant diarrhea, hemorrhagic colitis, thrombotic thrombocytic purpura, and hemolytic uremic syndrome in humans (17, 29, 31, 34, 41, 56). *E. coli* O157:H7 is the most common serotype isolated from individuals with hemorrhagic colitis. Outbreaks of infection with the *E. coli* O157:H7 have been associated with the consumption of beef products (4, 45, 49, 51) and raw milk (7, 37). *E. coli* O157:H7 and other SLT-EC serotypes that are pathogenic to humans have also been isolated from beef products (11, 12, 48, 61), raw milk (62), milk filters (10), and the feces of cattle with (5, 35, 42, 55) and without (7, 38, 62) diarrhea. This evidence suggests that cattle are a reservoir for SLT-EC and that raw milk and beef products are primary vehicles for human infection with these pathogens.

Several methods for biochemical identification and immunological detection of *E. coli* O157:H7 have been described (13, 32, 36, 44, 54, 57, 58); however, it is clear that SLT-EC from other serogroups are also associated with human infection (6, 29, 56). A more general method for detection of SLT-EC is based on Vero cell toxicity (VT) of broth culture supernatants (10, 33), polymixin B-treated bacterial cultures (28, 30), or fecal filtrates (28, 31, 50). Although VT assays have been applied to the detection of SLT-EC in clinical and food samples (10, 48, 62), they are time consuming. Immunological detection of SLTs may be more rapid than VT assays. However, there are several different antigenic variants or types of toxin (15, 20, 29, 56, 63); immunological methods are not likely to detect all SLTs with equal efficiency, and some SLTs may not be detected at all.

Genetic probes consisting of DNA fragments (39, 63) or synthetic oligonucleotides (8, 19, 27, 40, 52) have also been described for the detection of SLT-EC, and several recent reports have used the polymerase chain reaction (PCR) for this purpose (22, 24–26, 46, 47, 59). However, studies with the PCR have largely concentrated on differentiation among types or variants of SLT genes with DNA extracted from pure cultures of *E. coli* strains. PCR-based methods for detection of SLT-EC in food samples have not been adequately explored.

In this study, two sets of oligonucleotide primer pairs were used to amplify SLT gene targets; one pair amplifies DNA from *E. coli* that produce SLTI (9), and the second pair amplifies DNA from *E. coli* strains that produce SLTII (23) or types or variants of the SLTII family, namely, SLTIIc (53), VT2ha and VT2hb (21), SLTIIv(VTe) (18, 60), and SLTIIva (15). The PCR assay described in this study rapidly screens ground beef samples for SLT-EC.

MATERIALS AND METHODS

Bacterial strains, culture conditions, and media. The *E. coli* strains used in this study are shown in Table 1. These originated with M. Karmali (Hospital for Sick Children, Toronto, Ontario, Canada), M. Anand (Alberta Provincial Health Laboratory, Calgary, Alberta, Canada), M. Schoenderwoerd (Alberta Animal Health Laboratory, Edmonton, Alberta, Canada), C. Gyles (University of Guelph, Guelph, Ontario, Canada), and S. Scotland (Central Public Health Laboratories, London, England). Bacterial strains of other genera used in a panel for testing the specifity of PCR assays are presented in Table 2. The majority of these bacterial strains have been described elsewhere (16); however, in addition, *Campylobacter* strains from our own collection and *Vibrio* and *Yersinia* strains from M. Richter (Alberta Provincial Health Laboratory, Edmonton, Alberta, Canada) and M. Anand were included in the panel.

E. coli 319 (O157:H7), H30 (O26:H11), and B2F1/3 (O91:

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TABLE 1. Summary of SLT-PCR results with DNA from *E. coli* strains

Class and origin[a]	O serogroup	No. of strains	No. of strains with specific PCR products with SLT primer(s):		
			I	II	I+II
SLT-EC					
Human					
	1	1	1	1	1
	5	1	1	0	1
	26	13	13	0	13
	91	3	2	1	3
	103	5	0	5	5
	111	3	3	3	3
	121	1	0	1	1
	128	1	0	1	1
	145	2	1	1	2
	157	107	104	100	107
Bovine					
	5	1	1	0	1
	26	6	6	0	6
	111	8	8	1	8
Porcine					
	2	5	1	5	5
	107	2	1	2	2
	120	1	0	1	1
	121	1	0	1	1
	130	2	2	2	2
	138	1	0	1	1
	139	2	0	2	2
	141	1	0	1	1
	157	1	1	1	1
Non-SLT-EC					
Human EPEC	Various[b]	11	0	0	0
Human EIEC	124	1	0	0	0
Bovine ETEC[c]		15	0	0	0
Porcine ETEC[c]		13	0	0	0
E. coli K-12 strains		3	0	0	0

[a] EPEC, enteropathogenic *E. coli*; EIEC, enteroinvasive *E. coli*; ETEC, enterotoxigenic *E. coli*.

[b] One strain each from O serogroups 26, 55, 86, 111, 114, 119, 125, 126, 127, 128, and 142.

[c] Serotype not determined (K99 or K88 positive).

H21) were used for studies on the detection of SLT-EC in ground beef. Ground beef samples were cultured in modified Trypticase soy broth (mTSB) (43). MacConkey and Trypticase soy agar were used to enumerate bacteria.

Preparation of ground beef samples. The ground beef used in the study was purchased in a single lot from a local retail outlet. The ground beef had total aerobic plate counts of 4×10^4 bacteria per g and total coliform counts of 2×10^2 coliforms per g. SLT-EC were not detected in broth cultures of ground beef samples with SLT PCRs, nor was VT (33) detected in broth culture supernatants. Serial dilutions (10^{-5} to 10^{-10}) of overnight (16- to 18-h) cultures of each *E. coli* strain were made in mTSB, and 200 μl of each dilution was inoculated into ground beef samples. Bacterial counts of overnight cultures ranged from 1.7×10^9 to 4.8×10^8 CFU/ml, depending on the experiment and *E. coli* strain employed. Ground beef samples were mixed with mTSB (2 g in 18 ml or 25 g in 225 ml) and incubated at 42°C with aeration (180 rpm) for the time periods stated below. After incubation, a 1-ml aliquot of the culture was removed for enumeration of bacteria and DNA extraction. Three independent ground beef inoculation experiments were performed with *E. coli* 319 (O157:H7) and H30 (O26:H11), and two independent experiments were performed with *E. coli* B2F1/3 (O91:H21). The minimum number of *E. coli* CFU per gram in the initial ground beef inoculum, subsequently detected by the PCR assays, is presented as a range of the values from these experiments.

DNA extraction. Bacterial total DNA was prepared as previously described (2) and used for the optimization of PCR assays. Briefly, bacteria were grown overnight in brain heart infusion broth at 37°C with aeration. Bacterial cells were harvested by centrifugation and lysed with sodium lauryl sulfate. DNA was extracted from the lysate with cetyltrimethylammonium bromide-phenol-chloroform and precipitated with isopropanol. For PCR studies with cultures of ground beef, DNA was extracted by a procedure described previously for *Listeria monocytogenes* (16), which we have designated protocol I, and by a modification of this DNA extraction procedure, which we have designated protocol II. In protocol I, 0.3 ml of mTSB culture was mixed with 0.1 ml of lysis buffer (10 mM EDTA, 100 mM Tris [pH 8.0]) containing 7.5 mg of lysozyme per ml and 750 U of mutanolysin per ml. This suspension was incubated at 37°C for 30 min. A 0.1-ml aliquot of lysis buffer containing 5 mg of proteinase K per ml and 50 mg of Sarkosyl (sodium lauryl sarcosinate) per ml was next added to each sample, and the preparations were incubated for an additional 15 min at 37°C. DNA was precipitated from the lysate by adding 50 μl of 3 M sodium acetate and 1 ml of ice-cold absolute ethanol and collected by centrifugation. The pellet obtained was washed with 1 ml of 70% ethanol, air dried, resuspended in water, heated at 65°C for 30 min, and used in the PCR assays. In the modified DNA extraction procedure (protocol II), the lysozyme and mutanolysin treatment was omitted. Instead, 0.1 ml of lysis buffer, containing 5 mg of proteinase K per ml and 50 mg of Sarkosyl per ml, was mixed directly with 0.3 ml of each broth culture. This mixture was incubated for 10 min at 65°C. The DNA was precipitated from the lysate with ethanol and heat treated, as described above in protocol I, before use in the PCR assays. In some studies, 10 to 65 μl of broth culture was maintained at 100°C for 10 min and then used in the PCR assays. The latter preparations are referred to as boiled cultures (protocol III).

PCR assays. Oligonucleotide primers were synthesized by using a model 391 DNA synthesizer (PCR-Mate; Applied Biosystems) according to the protocol provided by the manufacturer. Oligonucleotide primers based on published nucleotide sequence data for *slt* genes (9, 15, 21, 23, 53, 60) were synthesized. The nucleotide sequence of each primer and the corresponding locations within SLTI and SLTII genes are given in Table 3. Amplification of bacterial DNA was performed in 100-μl volumes containing 2.0 to 4.0 mM $MgCl_2$, 10 mM Tris hydrochloride (pH 8.3); 50 mM KCl; template DNA; 0.2 mM (each) dATP, dGTP, dCTP, and dTTP (Pharmacia LKB Biotechnology, Inc.); 1 μM each primer; and 2.5 U of *Thermus aquaticus* (*Taq*) DNA polymerase (GIBCO/BRL Canada Ltd.). Amplifications were performed in a DNA thermal cycler (Perkin-Elmer Cetus) for 35 cycles with 1 min at 94°C, 1 min at 60°C, and 2 min at 72°C and a final extension at 72°C for 5 min. Negative (no template DNA) and positive (1 ng of purified genomic DNA from the *E. coli* O157 strain 319) controls were included with each ground beef inoculation experiment. After the PCR, 15-μl aliquots were analyzed by submarine gel electrophoresis with 1.0% agarose gels containing 0.25 μg of ethidium

TABLE 2. Bacterial strains from species other than *E. coli* that were negative in the SLT PCR

Species	No. of strains	Species	No. of strains
Aeromonas hydrophila	1	*Salmonella cholerae-suis*	1
Campylobacter fetus	1	*Serratia marcescens*	1
Campylobacter jejuni	1	*Vibrio cholerae*	1
Campylobacter intestinalis	1	*Vibrio parahaemolytica*	1
Campylobacter bubolus	1	*Vibrio vulnificus*	1
Enterobacter cloacae	1	*Yersinia enterocolitica*	4
Klebsiella pneumoniae	1	*Yersinia pseudotuberculosis*	3
Pasteurella haemolytica	1	*Listeria monocytogenes*	3
Proteus vulgaris	1	*Listeria innocua*	1
Pseudomonas aeruginosa	2	*Listeria ivanovii*	1
Pseudomonas fluorescens	1	*Micrococcus luteus*	1
Pseudomonas testosteroni	1	*Staphylococcus aureus*	4
Salmonella montevideo	1	*Streptococcus agalactiae*	1
Salmonella typhimurium	4	*Streptococcus faecalis*	3

bromide per ml. The DNA samples were analyzed by agarose gel electrophoresis (60 min at 100 V), visualized by UV transillumination, and photographed. Molecular size markers (100-bp ladder; Pharmacia LKB Biotechnology, Inc.) were included in each gel. SLTI and SLTII oligonucleotide primer pairs, used in combination, detected 10 pg of purified DNA from *E. coli* H30 (O26:H11; SLTI) and 1 pg of purified DNA from *E. coli* B2F1/3 (O91:H21; SLTII variants VT2ha and VT2hb) and 319 (O157:H7; SLTI and SLTII).

RESULTS

Specificity of SLT primers. SLTI oligonucleotide primers generated PCR products of the predicted size, ca. 600 bp, with DNA from SLTI-producing *E. coli* but not with DNA from *E. coli* strains that produce SLTII or variants of SLTII (Fig. 1). Similarly, SLTII oligonucleotide primers generated PCR products of the predicted size, ca. 800 bp, with DNA from SLTII- and SLTII variant-producing *E. coli* strains but not from those that produce SLTI. SLT primers generated PCR products with DNA from all SLT-EC strains tested (Table 1); however, no PCR products were obtained with DNA from *E. coli* strains that did not produce SLT or from 44 bacterial strains of 28 other species (Table 2).

Detection of SLT-EC in ground beef with PCR assays. When ground beef samples were cultured in mTSB (2 g in 18 ml or 25 g in 225 ml) for 4 h, an initial sample inoculum of 8 to 12 CFU of *E. coli* O157:H7 strain 319 per g was detected by SLT PCR with DNA extracted from broth cultures (Fig. 2). After 6 and 24 h of incubation, an initial inoculum of 0.8 to 1.2 CFU of this *E. coli* strain per g was detected.

Initial inocula of 1.4 to 1.5 and 4.8 to 5.5 CFU/g of *E. coli* B2F1/3 (O91:H21; VT2ha and VT2hb) and H30 (O26:H11; SLTI), respectively, were detected in 6-h mTSB cultures of ground beef samples with the SLT PCR (Fig. 3).

A higher level of detection of SLT-EC with the PCR was obtained with extracted DNA (protocols I and II) than with boiled cultures (Fig. 4). With boiled cultures, an initial sample inoculum of 80 to 120 CFU of *E. coli* O157:H7 strain 319 per g could be detected by the SLT PCR after 6 h of incubation in mTSB, compared with detection of 0.8 to 1.2 CFU/g for this strain with DNA extracted from the broth culture by protocol I or II. The modified DNA extraction procedure, protocol II, appeared to provide a sensitivity in SLT-EC detection that was equivalent to that of protocol I (16).

DISCUSSION

In this report a rapid and sensitive method for detection of SLT-EC in ground beef samples is described. The assay is based on a short period of selective culture followed by DNA extraction from bacterial growth and PCR-based amplification of target DNA with SLT oligonucleotide primers.

In the design of the SLT oligonucleotide primers, attempts were made to identify conserved regions within SLT genes by a comparison of the published nucleotide sequences of *slt*I (9), *slt*II (23), *slt*IIv (60), *slt*IIva (15), and *vt*2ha and *vt*2hb (21). Several base degeneracies would have had to have been incorporated into the sequences of the oligonucleotides to allow amplification of all SLT genes with one primer pair; however, degenerate SLT PCR primers such as these have

TABLE 3. Oligonucleotide primers used in the study

Primer	Oligonucleotide sequence (5′-3′)	% G+C	T_m[a] (°C)	Location within gene[b]	Predicted size of amplified product (bp)
SLTI-F	ACA CTG GAT GAT CTC AGT GG	50	71.2	938–957	
SLTI-R	CTG AAT CCC CCT CCA TTA TG	50	71.2	1539–1520	614
SLTII-F	CCA TGA CA[c]A CGG ACA GCA GTT	54.2	73.7	624–644	
SLTII-R	CCT GTC AA[d]C TGA GCA CTT TG	50	71.2	1403–1384	779

[a] The predicted melting temperatures (T_m) of the oligonucleotides were calculated by using the computer program PC/GENE (release 6.5; Intelligenetics, Mountain View, Calif.).

[b] The position numbers are those in the published nucleotide sequences of *slt*I (9) and *slt*II (23).

[c] G is in this position in *slt*IIva (15).

[d] G is in this position in *slt*IIv, *slt*IIva, and *slt*IIvhb (15, 21, 60).

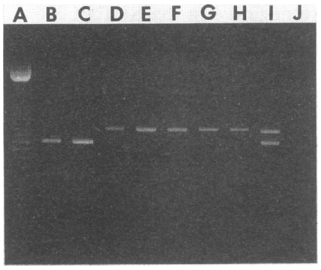

FIG. 1. Amplification of DNA from *E. coli* strains with SLT oligonucleotide primers. After DNA amplification, a 15-μl aliquot of each 100-μl PCR reaction mix was analyzed by agarose gel electrophoresis (1.0% agarose containing ethidium bromide). SLTI and SLTII oligonucleotide primers were used in combination. Lanes: A, molecular size markers (100-bp ladder); B through J, PCR products obtained with DNA from *E. coli* strains H19 (O26:H11; SLTI) (B), H30 (O26:H11; SLTI) (C), C600 (933w) (O?R:K12; SLTII) (D), 412 (O139:K82; SLTIIv) (E), H.I.8 (O128:B12; SLTIIva) (F), B2F1/3 (O91:H21; SLTII variants VT2ha and VT2hb [21]) (G), E32511 (O157:H$^-$; SLTII-c) (H), 319 (O157:H7; SLTI and SLTII) (I), and DH5α (O?R:K12; none; negative control) (J). DNA from *E. coli* H30 and C600 (933w) was used as a positive control for the SLTI and SLTII oligonucleotide primer pairs, respectively.

been used with apparent success (26). Instead, two sets of oligonucleotide primers were used, with the first pair homologous to *slt*I and the second pair homologous to *slt*II and *slt*II variants. This resulted in a one-base mismatch for each of the two SLTII primers from some *slt* variants (Table 3). This level of homology might provide high specificity and equivalent sensitivity among SLT genes in PCR detection. At the same time, sufficient differences exist between the

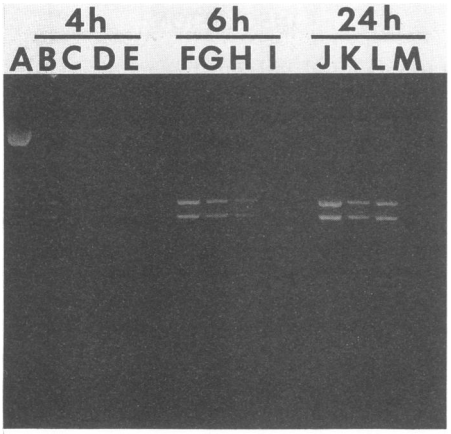

FIG. 2. Comparison of the PCR products obtained from mTSB cultures of ground beef inoculated with *E. coli* O157:H7 strain 319. Ground beef samples (2 g) were incubated for 4, 6, and 24 h with aeration in 18 ml of mTSB at 42°C. DNA was extracted from bacterial cultures, and PCR assays were carried out with SLTI and SLTII primer pairs (in combination). After DNA amplification, a 15-μl aliquot of each 100-μl PCR reaction mix was analyzed by agarose gel electrophoresis (1.0% agarose containing ethidium bromide). Lanes: A, molecular size markers (100-bp ladder); B through E, PCR products from cultures of ground beef inoculated with 8×10^2, 80, 8, and 0 CFU, respectively, of *E. coli* strain 319 per g and incubated for 4 h in mTSB; F through I and J through M, PCR products from cultures of ground beef inoculated with 80, 8, 0.8, and 0 CFU of *E. coli* 319 per g and incubated for 6 and 24 h, respectively, in mTSB.

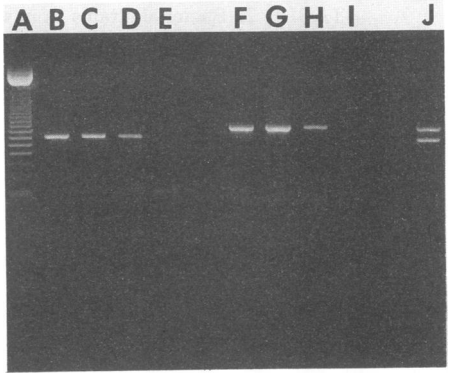

FIG. 3. Comparison of the PCR products obtained after amplification of DNA extracted from cultures of ground beef inoculated with *E. coli* B2F1/3 and H30. Ground beef samples (2 g) inoculated with each strain were grown for 6 h with aeration in 18 ml of mTSB at 42°C. After DNA extraction from bacterial cultures, DNA amplification was carried out with SLTI and SLTII oligonucleotide primers (in combination). A 15-μl aliquot of each 100-μl PCR reaction mix was analyzed by agarose gel electrophoresis (1.0% agarose with ethidium bromide). Lanes A, molecular size markers (100-bp ladder); B through E, initial sample inocula of 5.3×10^2, 53, and 0 CFU, respectively, of *E. coli* H30 per g; F through I, initial sample inocula of 1.5×10^2, 15, 1.5, and 0 CFU, respectively, of *E. coli* B2F1/3 per g; J, PCR product obtained with SLT primers and 1 ng of DNA extracted from an mTSB culture of *E. coli* 319 (positive control).

SLTI and SLTII oligonucleotide primer sets to allow them to be used individually to differentiate between *slt*I and genes of the *slt*II family. In this respect, the oligonucleotide primer pairs are similar to those described by Pollard et al. (46), with the exception that variants such as *slt*IIv are also detected. This was felt to be desirable, since *E. coli* that produce SLTIIv or SLTIIv variants may indeed be involved in human disease; e.g., strain H.I.8 (O128:B12), which produces SLTIIva (15), was isolated from an infant with diarrhea (33). Although *E. coli* strains that produce this and other SLT variants may be involved in a minority of clinical cases in humans, it would seem that no satisfactory answers can be obtained concerning their importance if methods of analysis are designed to exclude them from detection. Although the PCR is a multiplex, the primary goal is to detect all *slt* genes and not to differentiate among types and variants. Differentiation among SLT genes, after detection with this assay, can be accomplished by several secondary procedures. These include a multiplex PCR with specific primer pairs that differentiate among genes of the *slt*II family, restriction endonuclease digestion of *slt*II family PCR products (24, 25, 59), and hybridization studies with specific oligonucleotide probes (19).

A high sensitivity of detection was achieved by culture of ground beef samples before the PCR. A 6-h incubation in mTSB at 42°C with aeration allowed detection of approximately 1 CFU of the SLT-EC strains tested per g. The time needed for detection, including sample preparation, DNA extraction, PCR, and agarose gel electrophoresis, is approximately 9 h. This method is therefore more rapid and sensitive than VT assays (33), enzyme-linked immunosorbent assays (1, 3, 43), DNA hybridization (8, 19, 27, 39, 40, 52, 63), and direct-sample PCR (14) methods.

While it is possible that overnight (16- to 18-h) *E. coli* cultures may contain a significant amount of DNA from

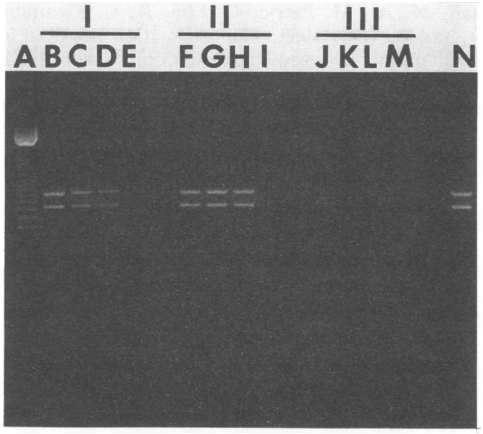

FIG. 4. PCR products obtained after amplification with DNA obtained by three different methods from bacterial cultures of ground beef inoculated with *E. coli* O157:H7 strain 319. Ground beef samples (2 g) were grown for 6 h with aeration in 18 ml of mTSB at 42°C. DNA was extracted from the cultures as described in Materials and Methods. PCRs with SLT primers were performed with DNA purified by protocol I, a modification of this procedure (protocol II), and boiled bacterial cultures (protocol III). After DNA amplification, a 15-μl aliquot of each 100-μl PCR reaction mix was analyzed by agarose gel electrophoresis (1.0% agarose containing ethidium bromide). Lanes: A, molecular size markers (100-bp ladder); B through E, F through I, and J through M, PCR products obtained with DNA extracted from cultures of ground beef inoculated with 1.2×10^3, 1.2×10^2, 1.2×10, and 0 CFU, respectively, of *E. coli* 319 per g by methods I, II, and III (as indicated); N, PCR product obtained with SLT primers and 1 ng of DNA extracted from an mTSB culture of *E. coli* 319 (with no ground beef).

nonviable cells, and this DNA may have been inoculated with viable cells into the ground beef samples, the data obtained from these experiments suggest that this did not introduce substantial error into the estimates of the number of SLT-EC detected in PCR assays. At least 4 to 6 h of culture is required for optimal PCR detection of low numbers of CFU in the initial ground beef inoculum. In addition, as the culture proceeded, PCR product bands became stronger and PCR products appeared in samples with low inocula. This would suggest that live and not dead cells were responsible for positive results in these SLT PCR assays.

The modified DNA extraction procedure, protocol II, appeared to have the same level of sensitivity in SLT-EC detection as protocol I, a DNA extraction procedure which we have used to obtain DNA from *L. monocytogenes* strains (16). This modification reduced the number of manipulations required in DNA extraction and decreased DNA preparation times from approximately 2 h to 1 h. The modified DNA extraction procedure omitted treatment of bacterial suspensions with lysozyme and mutanolysin, a step required in protocol I. Both of these enzymes are thought to aid in bacterial lysis by degradation of the peptidoglycan layer of the cell wall. Since the peptidoglycan layer is less substantial in gram-negative bacteria than in gram-positive bacteria, it is possible that exclusion of these enzymes not only saves time but also results in a lysis procedure that is somewhat selective for gram-negative bacteria. Although boiling of bacterial cultures provided satisfactory results with high concentrations of SLT-EC, the boiled-sample method was less sensitive than the modified DNA extraction procedure for PCR detection of SLT-EC at low concentrations. Boiled material also produced multiple bands in some PCR experiments (data not shown).

One important shortfall in the procedure that we have described is that information such as the serotype cannot be obtained until the SLT-producing strain is isolated from the broth culture. It is also possible that one or more types of SLT-EC may be present in the samples. The primary use of this procedure is therefore to identify food samples that contain SLT-EC. Methods such as selection on MacConkey-sorbitol agar for *E. coli* O157:H7 strains, DNA hybridization with *slt* probes, or enzyme-linked immunosorbent assays may be useful in the isolation of SLT-EC in samples found positive in this screening assay. However, we are also exploring procedures that would allow direct characterization of SLT-EC isolates with the PCR. One of these includes PCR-based detection of relatively serotype-specific targets for common SLT-producing pathogens such as *E. coli* O157:H7.

ACKNOWLEDGMENTS

This research was supported by Short Start Funding from the Food Production and Inspection Branch of Agriculture Canada.

We thank H. Cho for synthesizing the oligonucleotide primers.

REFERENCES

1. **Ashkenazi, S., and T. G. Cleary.** 1989. Rapid method to detect Shiga toxin and Shiga-like toxin I based on binding to globotriosyl ceramide (GB_3), their natural receptor. J. Clin. Microbiol. **27**:1145–1150.
2. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1987. Current protocols in molecular biology, p. 2.4.1–2.4.2. John Wiley & Sons, Inc., New York.
3. **Basta, M., M. Karmali, and C. Lingwood.** 1989. Sensitive receptor-specified enzyme-linked immunosorbent assay for *Escherichia coli* verocytotoxin. J. Clin. Microbiol. **27**:1617–1622.
4. **Belongia, E. A., K. L. MacDonald, G. L. Parham, K. E. White, J. A. Korlath, M. N. Lobato, S. M. Strand, K. A. Casale, and M. T. Osterholm.** 1991. An outbreak of *Escherichia coli* O157:H7 colitis associated with consumption of precooked meat patties. J. Infect. Dis. **164**:338–343.
5. **Blanco, J., E. A. Gonzalez, S. Garcia, M. Blanco, B. Regueiro, and S. Bernardez.** 1988. Production of toxins by *Escherichia coli* strains isolated from calves with diarrhea in Galicia (Northern Spain). Vet. Microbiol. **18**:297–311.
6. **Bopp, C. A., K. D. Greene, F. Pouch Downes, E. G. Sowers, J. G. Wells, and I. K. Wachsmuth.** 1987. Unusual verotoxin-producing *Escherichia coli* associated with hemorrhagic colitis. J. Clin. Microbiol. **25**:1486–1489.
7. **Borczyk, A. A., M. A. Karmali, H. Lior, and L. M. C. Duncan.** 1987. Bovine reservoir for verotoxin producing *Escherichia coli*. Lancet **i**:98.
8. **Brown, J. I., S. Sethabutr, M. P. Jackson, P. Lolekha, and P. Echeverria.** 1989. Hybridization of *Escherichia coli* producing Shiga-like toxin I, Shiga-like toxin II, and a variant of Shiga-like toxin I with synthetic oligonucleotide probes. Infect. Immun. **57**:2811–2814.
9. **Calderwood, S. B., F. Auclair, A. Donohue-Rolfe, G. T. Keusch, and J. J. Mekalanos.** 1987. Nucleotide sequence of the Shiga-like toxin genes of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **84**:4364–4368.
10. **Clarke, R. C., S. A. McEwan, V. P. Gannon, H. Lior, and C. L. Gyles.** 1989. Isolation of verocytotoxin-producing *Escherichia coli* from milk filters in south-western Ontario. Epidemiol. Infect. **102**:253–260.
11. **Doyle, M. P.** 1991. *Escherichia coli* O157:H7 and its significance in foods. Int. J. Food Microbiol. **12**:289–302.
12. **Doyle, M. P., and J. L. Schoeni.** 1987. Isolation of *Escherichia coli* O157:H7 from retail fresh meats and poultry. Appl. Envi-

ron. Microbiol. **53**:2394–2396.
13. **Farmer, J. J., and B. R. Davis.** 1985. H7 antiserum-sorbitol fermentation medium for detecting *Escherichia coli* O157:H7 associated with hemorrhagic colitis. J. Clin. Microbiol. **22**:620–625.
14. **Frankel, G., L. Riley, A. Giron, J. Valmassoi, A. Friedmann, N. Strockbine, S. Falkow, and G. K. Schoolnik.** 1990. Detection of *Shigella* in feces using DNA amplification. J. Infect. Dis. **161**:1252–1256.
15. **Gannon, V. P. J., C. Teerling, S. Masri, and C. L. Gyles.** 1990. Molecular cloning and nucleotide sequence of another variant of the *Escherichia coli* shiga-like toxin II family. J. Gen. Microbiol. **136**:1125–1135.
16. **Golsteyn Thomas, E. J., R. K. King, J. Burchak, and V. P. J. Gannon.** 1991. Sensitive and specific detection of *Listeria monocytogenes* in milk and ground beef with the polymerase chain reaction. Appl. Environ. Microbiol. **57**:2576–2580.
17. **Griffin, P. M., S. M. Ostroff, R. V. Tauxe, K. D. Greene, J. G. Wells, J. H. Lewis, and P. A. Blake.** 1988. Illness associated with *Escherichia coli* O157:H7 infections: a broad clinical spectrum. Ann. Intern. Med. **109**:705–712.
18. **Gyles, C. L., S. A. De Grandis, C. Mackenzie, and J. L. Brunton.** 1988. Cloning and nucleotide sequence analysis of the genes determining verocytotoxin production in a porcine edema disease isolate of *Escherichia coli*. Microb. Pathog. **5**:419–426.
19. **Hii, J., C. L. Gyles, T. Morooka, M. Karmali, R. Clarke, S. DeGrandis, and J. L. Brunton.** 1991. Development of Verotoxin 2- and Verotoxin 2 variant (VT2v)-specific oligonucleotide probes on the basis of the nucleotide sequence of the B cistron of VT2v from *Escherichia coli* E32511 and B2F1. J. Clin. Microbiol. **29**:2704–2709.
20. **Ito, H., M. Nishibuchi, and Y. Takeda.** 1991. Analysis of the antigenic differences between Verotoxin 2 (VT2) and VT2 (VT2vh) of Verotoxin-producing *Escherichia coli* by site directed mutagenesis. FEMS Microbiol. Lett. **79**:27–30.
21. **Ito, H., A. Terai, Y. Takeda, and M. Nishibuchi.** 1990. Cloning and nucleotide sequencing of vero toxin 2 variant genes from *Escherichia coli* O91:H21 isolated from a patient with hemolytic uremic syndrome. Microb. Pathog. **8**:47–60.
22. **Jackson, M. P.** 1991. Detection of Shiga toxin-producing *Shigella dysenteriae* type 1 and *Escherichia coli* using the polymerase chain reaction with incorporation of digoxigenin-11-dUTP. J. Clin. Microbiol. **29**:1910–1914.
23. **Jackson, M. P., R. J. Neill, A. D. O'Brien, R. K. Holmes, and J. W. Newland.** 1987. Nucleotide sequence analysis and comparison of the structural genes for Shiga-like toxin I and Shiga-like toxin II encoded by bacteriophages from *Escherichia coli* 933. FEMS Microbiol. Lett. **44**:109–114.
24. **Johnson, W. M., D. R. Pollard, H. Lior, S. D. Tyler, and K. R. Rozee.** 1990. Differentiation of genes coding for *Escherichia coli* Verotoxin type 2 and the Verotoxin associated with porcine edema disease (VTe) by the polymerase chain reaction. J. Clin. Microbiol. **28**:2351–2353.
25. **Johnson, W. M., S. D. Tyler, G. Wang, and H. Lior.** 1991. Amplification by the polymerase chain reaction of a specific target sequence in the coding for *Escherichia coli* verotoxin (VTe) variant. FEMS Microbiol. Lett. **84**:227–230.
26. **Karch, H., and T. Meyers.** 1989. Single primer pair for amplifying segments of distinct Shiga-like toxin genes by polymerase chain reaction. J. Clin. Microbiol. **27**:2751–2757.
27. **Karch, H., and T. Meyers.** 1989. Evaluation of oligonucleotide probes for identification of Shiga-like-toxin-producing *Escherichia coli*. J. Clin. Microbiol. **27**:1180–1186.
28. **Karmali, M. A.** 1987. Laboratory diagnosis of verotoxin-producing *Escherichia coli* infections. Clin. Microbiol. Newsl. **9**:65–70.
29. **Karmali, M. A.** 1989. Infection by verocytotoxin-producing *Escherichia coli*. Clin. Microbiol. Rev. **2**:15–38.
30. **Karmali, M. A., M. Petric, C. Lim, R. Cheung, and G. S. Arbus.** 1985. Sensitive method for detecting low numbers of Verotoxin-producing *Escherichia coli* in mixed cultures by use of colony sweeps and polymixin extraction of verotoxins. J. Clin. Microbiol. **22**:614–619.
31. **Karmali, M. A., M. Petric, C. Lim, R. C. Flemming, G. S. Arbus, and H. Lior.** 1985. The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing *Escherichia coli*. J. Infect. Dis. **151**:775–782.
32. **Kleanthous, N., R. Fry, H. R. Smith, R. J. Gross, and B. Rowe.** 1988. The use of sorbitol-MacConkey in conjunction with specific antiserum for the detection of Vero cytotoxin-producing strains of *Escherichia coli* O157. Epidemiol. Infect. **101**:327–335.
33. **Konowalchuk, J., J. L. Spiers, and S. Starvic.** 1977. Vero response to a cytotoxin of *Escherichia coli*. Infect. Immun. **18**:775–779.
34. **Kovacs, M. J., J. Roddy, S. Gregoire, W. Cameron, L. Eidus, and J. Drouin.** 1990. Thrombotic thrombocytic purpura following hemorrhagic colitis due to *Escherichia coli* O157:H7. Am. J. Med. **88**:177–179.
35. **Mainil, J. G., C. J. Duchesnes, S. C. Whipp, L. R. Marques, A. D. O'Brien, T. A. Casey, and H. W. Moon.** 1987. Shiga-like toxin production and attaching and effacing activity of *Escherichia coli* associated with calf diarrhea. Am. J. Vet. Res. **48**:743–748.
36. **March, S. B., and S. Ratnam.** 1986. Sorbitol-MacConkey medium for detection of *Escherichia coli* associated with hemorrhagic colitis. J. Clin. Microbiol. **23**:869–972.
37. **Martin, M. L., L. D. Shipman, J. G. Wells, M. E. Potter, K. Hedberg, I. K. Wachsmuth, R. V. Tauxe, J. P. Davis, J. Arnoldi, and J. Tilleli.** 1986. Isolation of *Escherichia coli* O157:H7 from dairy cattle associated with two cases of haemolytic uraemic syndrome. Lancet **ii**:1043.
38. **Montenegro, M. A., M. Bülte, T. Trumpf, S. Aleksić, G. Reuter, R. Bulling, and R. Helmuth.** 1990. Detection and characterization of fecal Verotoxin-producing *Escherichia coli* from healthy cattle. J. Clin. Microbiol. **28**:1417–1421.
39. **Newland, J. W., and R. J. Neill.** 1988. DNA probes for Shiga-like toxins I and II and for toxin converting bacteriophages. J. Clin. Microbiol. **26**:1292–1297.
40. **Notermans, S., K. Wernars, P. S. Soentoro, J. Dufrenne, and W. Jansen.** 1991. DNA hybridization and latex agglutination for detection of heat-labile- and shiga-like toxin-producing *Escherichia coli* in meat. Int. J. Food Microbiol. **13**:31–40.
41. **O'Brien, A. D., and R. K. Holmes.** 1987. Shiga and Shiga-like toxins. Microbiol. Rev. **51**:206–220.
42. **Orskov, F., I. Orskov, and J. A. Villar.** 1987. Cattle as a reservoir of verotoxin-producing *Escherichia coli*. Lancet **ii**: 276.
43. **Padhye, N. V., and M. P. Doyle.** 1991. Rapid procedure for detecting enterohemorrhagic *Escherichia coli* O157:H7 in food. Appl. Environ. Microbiol. **57**:2693–2698.
44. **Padhye, N. V., and M. P. Doyle.** 1991. Production and characterization of a monoclonal antibody specific for enterohemorrhagic *Escherichia coli* O157:H7 and O26:H11. J. Clin. Microbiol. **29**:99–103.
45. **Pai, C. H., R. Gordon, H. V. Sims, and L. E. Bryan.** 1984. Sporadic cases of hemorrhagic colitis associated with *Escherichia coli* O157:H7; clinical, epidemiological and bacteriological features. Ann. Intern. Med. **101**:738–742.
46. **Pollard, D. R., W. M. Johnson, H. Lior, S. D. Tyler, and K. R. Rozee.** 1990. Rapid and specific detection of Verotoxin genes in *Escherichia coli* by the polymerase chain reaction. J. Clin. Microbiol. **28**:540–545.
47. **Pollard, D. R., W. M. Johnson, H. Lior, S. D. Tyler, and K. R. Rozee.** 1990. Differentiation of Shiga toxin and verocytotoxin type I genes by the polymerase chain reaction. J. Infect. Dis. **162**:1195–1198.
48. **Read, S. C., C. L. Gyles, R. C. Clarke, H. Lior, and S. McEwan.** 1990. Prevalance of verocytotoxigenic *Escherichia coli* in ground beef, pork and chicken in southwestern Ontario. Epidemiol. Infect. **105**:11–20.
49. **Riley, L. W., R. S. Remis, S. D. Helgerson, H. B. McGee, J. G. Wells, B. R. Davis, R. J. Herbert, E. S. Olcott, L. M. Johnson, N. T. Hargett, P. A. Blake, and M. L. Cohen.** 1983. Haemorrhagic colitis associated with a rare serotype of *Escherichia coli*. N. Engl. J. Med. **308**:681–685.

50. **Ritchie, M., S. Partington, J. Jessop, and M. T. Kelly.** 1992. Comparison of a direct fecal Shiga-like toxin assay and sorbitol-MacConkey agar culture for laboratory diagnosis of enterohemorrhagic *Escherichia coli* infection. J. Clin. Microbiol. **30:**461–464.
51. **Ryan, C. A., R. V. Tauxe, G. W. Hosek, J. G. Wells, H. W. Stoesz, P. W. McFadden, Jr., P. W. Smith, G. F. Wright, and P. A. Blake.** 1986. *Escherichia coli* O157:H7 diarrhea in a nursing home: clinical, epidemiological and pathological findings. J. Infect. Dis. **154:**631–638.
52. **Samadpour, M., J. Liston, J. E. Ongerth, and P. I. Tarr.** 1990. Evaluation of DNA probes for detection of Shiga-like toxin producing *Escherichia coli* in foods and calf fecal samples. Appl. Environ. Microbiol. **56:**1212–1215.
53. **Schmitt, C. K., M. L. McKee, and A. D. O'Brien.** 1991. Two copies of Shiga-like toxin II-related genes common in enterohaemorrhagic *Escherichia coli* strains are responsible for the antigenic heterogeneity of the O157:H$^-$ strain E32511. Infect. Immun. **59:**1065–1073.
54. **Scotland, S. M., T. Cheasty, A. Thomas, and B. Rowe.** 1991. Beta-glucuronide activity of *Escherichia coli*, including serogroup O157, isolated in the United Kingdom. Lett. Appl. Microbiol. **13:**42–44.
55. **Sherwood, D., D. Snodgrass, and A. D. O'Brien.** 1985. Shiga-like toxin production from *Escherichia coli* associated with calf diarrhea. Vet. Rec. **116:**217–218.
56. **Smith, H. R., and S. M. Scotland.** 1988. Vero cytotoxin-producing *Escherichia coli*. J. Med. Microbiol. **26:**77–85.
57. **Thompson, J. S., D. S. Hodge, and A. A. Borczyk.** 1990. Rapid biochemical test to identify verocytotoxin-positive strains of *Escherichia coli* serotype O157:H7. J. Clin. Microbiol. **28:**2165–2168.
58. **Todd, E. C. D., R. A. Szabo, P. Peterkin, A. N. Sharpe, L. Parrington, D. Bundle, M. A. J. Gidney, and M. B. Perry.** 1988. Rapid hydrophobic grid membrane filter-enzyme-labeled antibody procedure for identification and enumeration of *Escherichia coli* O157:H7 in foods. Appl. Environ. Microbiol. **54:**2536–2540.
59. **Tyler, S. D., W. M. Johnson, H. Lior, G. Wang, and K. R. Rozee.** 1991. Identification of Verotoxin type 2 variant B subunit genes in *Escherichia coli* by the polymerase chain reaction and restriction length polymorphism analysis. J. Clin. Microbiol. **29:**1339–1343.
60. **Weinstein, D. L., M. P. Jackson, J. E. Samuel, R. K. Holmes, and A. D. O'Brien.** 1988. Cloning and sequencing of a Shiga-like toxin type II variant from an *Escherichia coli* strain responsible for edema disease of swine. J. Bacteriol. **170:**4223–4230.
61. **Wells, J. G., B. R. Davis, I. K. Wachsmuth, L. W. Riley, R. S. Remis, R. Sokolow, and G. K. Morris.** 1983. Laboratory investigation of hemorrhagic colitis outbreaks associated with a rare *Escherichia coli* serotype. J. Clin. Microbiol. **18:**512–520.
62. **Wells, J. G., L. D. Shipman, K. D. Greene, E. G. Sowers, J. H. Green, D. N. Cameron, F. P. Downes, M. L. Martin, P. M. Griffin, S. M. Ostroff, M. E. Potter, R. V. Tauxe, and I. K. Wachsmuth.** 1991. Isolation of *Escherichia coli* O157:H7 and other Shiga-like toxin producing *E. coli* from dairy cattle. J. Clin. Microbiol. **29:**985–989.
63. **Willshaw, G. A., H. R. Smith, S. M. Scotland, A. M. Field, and B. Rowe.** 1987. Heterogeneity of *Escherichia coli* phages encoding Vero cytotoxins: comparison of cloned sequences determining VT1 and VT2 and development of specific gene probes. J. Gen. Microbiol. **133:**1309–1317.