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Multiplex PCR for Detection of the Heat-Labile Toxin Gene and Shiga-Like Toxin I and II Genes in *Escherichia coli* Isolated from Natural Waters

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A triplex PCR method was developed to simultaneously amplify a heat-labile toxin sequence (LT) of 258 bp, a shiga-like toxin I sequence (SLT I) of 130 bp, and a shiga-like toxin II sequence (SLT II) of 346 bp from toxigenic strains of *Escherichia coli*. This method was used to screen 377 environmental *E. coli* isolates from marine waters or estuaries located in Southern California and North Carolina for enterotoxigenic or enterohemorrhagic *E. coli* strains. Of the 377 *E. coli* screened, one isolate was found to belong to the enterotoxigenic group, since it contained a LT homologous sequence, and one isolate was found to belong to the enterohemorrhagic group, since it contained a SLT I homologous sequence. None was found to contain SLT II homologous sequences. The pathogenicity of the positive environmental *E. coli* isolates was confirmed by standard bioassays with Y-1 adrenal cells and Vero cells to confirm toxin production. Our results suggest that toxigenic *E. coli* occurs infrequently in environmental waters and that there is a low public health risk from toxigenic *E. coli* in coastal waters.

Although most *Escherichia coli* strains are harmless commensals in the human gut, some strains are known to cause disease. Two groups that contain strains with the ability to cause disease are the enterotoxigenic *E. coli* (ETEC) and enterohemorrhagic *E. coli* (EHEC). ETEC strains are a frequent cause of diarrheal disease throughout the world. These organisms contain genes which carry the genetic information for the production of heat-labile (LT) enterotoxin and heat-stable (ST) enterotoxin (9). The ETEC strains that elaborate these toxins typically cause profuse, watery diarrhea and are often implicated in cases of traveler's diarrhea (5). EHEC strains may cause hemorrhagic colitis and hemolytic uremic syndrome (13). These organisms produce potent cytotoxins similar to Shiga toxin (8). The clinical symptoms include bloody and copious diarrhea unaccompanied by fecal leukocytes and can be seen in afebrile patients (7). These features distinguish it from classic dysentery due to *Shigella* spp. or enteroinvasive *E. coli* which are characterized by high fever, toxemia, and scanty stools of blood and mucus containing many fecal leukocytes. A Shiga-like toxin I and II (SLT I and SLT II) have been identified as virulence factors in EHEC organisms (8, 9).

Both ETEC and EHEC infections have been associated with the ingestion of food or water contaminated with these organisms (4, 8, 14). One recent outbreak, in Cabool, Mo., was directly attributed to ingestion of *E. coli* O157:H7-contaminated water from the public water system (5, 15). Swerdlow et al. (15) concluded that cities using untreated water incur a greater risk of widespread illness from contaminated drinking water. The risks of contracting ETEC and EHEC infections from contaminated recreational waters have not been clearly established (14). However, a recent study of pathogenic *E. coli* in freshwater environments indicates that environmental waters may be an important reservoir for infectious *E. coli* (6).

To better determine the health risks associated with exposure to pathogenic *E. coli* in the environment, the frequency at which pathogenic *E. coli* occurs in the environment must be determined. The standard bioassays used for identification of pathogenic *E. coli*, such as cytopathic effects on Y-1 adrenal cells and rabbit ileal loop, are not readily adaptable for screening large numbers of *E. coli* isolates. Bioassays are labor intensive, costly, and can take several weeks to obtain results. The development of molecular technologies and their applications in the environmental field have allowed researchers to better address public health concerns, since molecular identification of microorganisms from water samples provides a more rapid and sensitive alternative to traditional detection methodologies. Recently, restriction fragment length polymorphisms were used to demonstrate that an *Enterobacter cloacae* isolated from the New Haven County, Conn., water distribution system was not responsible for the *E. cloacae* infections at local hospitals (3).

PCR using two or more primers to amplify two or more target sequences, called multiplex PCR, and subsequent DNA hybridization have been applied to environmental samples for the detection and differentiation of coliforms. Bej et al. (2) used multiplex PCR and DNA hybridization to identify coliform bacteria in water samples. More recently, Tsai et al. (16) demonstrated that PCR and DNA hybridization could be used to detect *E. coli* in sewage and sludge samples. In this study, a triplex PCR method was developed to simultaneously screen *E. coli* for SLT I, SLT II, and LT homologous sequences. A random sample of 377 *E. coli* isolates obtained from marine waters was examined for the presence of ETEC and EHEC homologous sequences. Three sets of primers and three internal probes were used to amplify and hybridize LT, SLT I, and SLT II homologous sequences in *E. coli* cells isolated from the

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marine environment for rapid identification of disease-causing strains.

MATERIALS AND METHODS

Bacterial isolates. One hundred eighty-six surfzone and 63 deep-water *E. coli* isolates were obtained from Southern California ocean waters. The deep-water isolates were collected 5 miles offshore in 30 m of water at a treated sewage effluent outfall discharge site. One hundred twenty-eight *E. coli* strains were isolated from a North Carolina estuary that is closed to shellfish harvesting because of high coliform counts. Eleven of the North Carolina isolates were from tissues of oysters living in the closed estuary.

All water samples were collected in sterile bottles, kept on ice, and processed within 6 h. Twenty-two of the California surfzone *E. coli* isolates were obtained by using Colilert-Marine Water media (Environetics, Branford, Conn.) as previously described (11). The remainder of the *E. coli* isolates were isolated by membrane filtration. Briefly, 100 ml of marine water was filtered through a 0.45-μm-pore-size filter (Gelman Sciences, Ann Arbor, Mich.), the filters were placed on either m-Tec (Difco, Detroit, Mich.) or Tergitol 7 (Becton Dickinson, Cockeysville, Md.) agar and incubated at either 37 or 44.5°C. Presumptive *E. coli* isolates were subsequently streaked onto a quadplate containing Simmons citrate agar (Difco), tryptic soy agar (Difco), MacConkey agar (Difco), and EC-MUG agar (Difco) and incubated at 37°C for 24 h. Growth results and fluorescence were recorded for each bacterial isolate. All organisms were biochemically identified with the API 20E identification system or a Vitek Jr. (bioMerieux Vitek Inc., Hazelwood, Mo.).

American Type Culture Collection (ATCC) *E. coli* strains and strains of other genera were used as positive and negative controls. The six ATCC strains used for controls (and the ETEC or EHEC toxins which they produce) are ATCC 35401 (LT and ST), ATCC 43886 (LT), ATCC 43889 (SLT II), ATCC 43890 (SLT I), ATCC 43895 (SLT I and SLT II), and ATCC 43894 (SLT I and SLT II). *Escherichia fergusonii*, *Escherichia hermannii*, *Klebsiella pneumoniae*, *Klebsiella oxetoca*, *Vibrio cholerae* non-O1, *Vibrio damsela*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Enterobacter agglomerans*, two *Enterobacter cloacae*, and six gram-negative bacterial isolates that could not be identified by either the Vitek or the API 20E database were used as negative controls to ensure primer and probe specificity.

DNA isolation. Bacterial DNA was isolated by a modification of a method described by Woo et al. (18). A 1-μl inoculating loop was used to remove 2 to 5 mg of *E. coli* from the tryptic soy agar panel of the quadplate. The cells were suspended in a mixture of 135 μl of TNE (10 mM Tris-HCl [pH 8.0], 10 mM NaCl, 10 mM EDTA [pH 8.0], and 135 μl of TNET (10 mM Tris-HCl [pH 8.0], 10 mM NaCl, 10 mM EDTA [pH 8.0], 2% Triton X-100). The cell suspensions were digested for 30 min at 37°C with 30 μl of freshly prepared lysozyme (5 mg/ml) made in TNE. Fifteen microliters of proteinase K (20 mg/ml in 10 mM Tris-HCl [pH 7.5]) was added, and the suspensions were incubated for 2 h at 65°C. The resulting cell lysates were prepared for use as PCR templates by diluting 10 μl of cell lysate with 90 μl of sterile deionized water.

Triplex PCR parameters for toxigenic *E. coli* amplification. PCR parameters and probe hybridization conditions were developed by using the seven ATCC *E. coli* strains known to be positive for ETEC and EHEC toxin production as standards. The primers used to amplify SLT I (5′-GAAGAGTCCGTGG*GATTACG-3′* [T_m = 62.5°C] and 5′-AGCGATGCAGCTAT TAATAA-3′ [T_m = 54.5°C]) and SLT II (5′-TTAACCACAC CCACGGCAGT-3′ [T_m = 62.5°C] and 5′-GCTCTGGATGC ATCTCTGGT-3′ [T_m = 62.5°C]) were first described by Pollard et al. (12). Three LT sequences were located in the GenBank database (accession numbers M35581, M57244, and K01995) and were aligned by using the PC-Gene (Intelligenetics, Mountain View, Calif.) multiple sequence alignment subroutine. Using the consensus sequence, two PCR primers (5′-TGTTTCCACTTCTCTTAG-3′ [T_m = 50.5°C] and 5′-TATTCCCTGTTACGATGT-3′ [T_m = 50.5°C]) were designed for the amplification of the three LT sequences. The expected sizes for PCR-amplified products were 130, 346, and 258 bp for the SLT I, SLT II, and LT primers, respectively. All PCR primers and oligonucleotide probes were synthesized on an ABI 392 DNA/RNA Synthesizer (Applied Biosystems, Foster City, Calif.). All six primers were added to the reaction mixture at a final concentration of 0.3 μM each. Reaction mixtures were made by using the GeneAmp PCR kit (Perkin Elmer, Norwalk, Conn.) with native *Taq* polymerase as per the manufacturer's directions. Triplex PCR was performed on the diluted cell lysates by using the GeneAmp PCR System 9600 (Perkin Elmer). PCR parameters for amplification were as follows: 5 min at 94°C; 1.5 min at 94°C, 1.0 min at 45°C, 1.0 min at 72°C for 30 cycles; 7 min at 72°C; hold at 4°C.

PCR parameters for *uidA* amplification. The *uidA* gene encodes for the enzyme β-glucuronidase, an enzyme commonly used to differentiate *E. coli* from other coliforms. Two PCR primers were designed to amplify a 1.7-kb region of the *uidA* gene (5′-ATCAGCGTTGGTGGGAAAGC-3′ and 5′-TGCCAGTCCAGCGTTTTTGC-3′). The primers were designed with Oligo 4.1 software (National Biosciences, Plymouth, Minn.). These primers were used to demonstrate that the DNA extracts were able to serve as a template for PCR. PCR conditions and parameters were identical to those used for triplex PCR.

Dot blot and Southern analysis. PCR products were dot blotted in triplicate and screened by using internal oligonucleotide probes (LT, 5′-TACAGCCCTCACCCATATGAACA-3′; SLT I, 5′-CCACTCTGGGGGCAATTCTGATGCG-3′, and SLT II, 5′-CGTGTCGCAGCGCTGGAACGTTCCG-3′) that are homologous to the expected LT, SLT I, or SLT II-amplified products. Internal probes were developed by using Oligo 4.1 software. Briefly, 10 μl of PCR product was diluted in 90 μl of deionized water and denatured in the thermocycler (15 min at 95°C, and then hold at 4°C for 3 min). One hundred microliters of 20× SSC (1× SSC is 150 mM NaCl plus 15 mM sodium citrate [pH 7.0]) was added to the denatured PCR products and immediately blotted onto a Hybond-N+ membrane (Amersham, Arlington Heights, Ill.) with a Minifold I filtration apparatus (Schleicher & Schuell, Keene, N.H.). The DNA was UV cross-linked for 2 min with a Stratalinker 2400 (Stratagene, La Jolla, Calif.). Oligonucleotide probes were labeled by using the Genius 5 kit (Boehringer Mannheim, Indianapolis, Ind.). Membranes were prehybridized for 2 h at 50°C in hybridization solution (5× SSC, 0.1% *N*-lauroylsarcosine, 0.02% sodium dodecyl sulfate [SDS], 2% blocking reagent [Boehringer Mannheim]) and hybridized with labeled LT, SLT I, or SLT II probe (4 pmol/ml) for 1 h at 50°C. The membranes were washed twice in 2× SSC–0.1% SDS at 50°C for 15 min and twice in 0.5× SSC–0.1% SDS for 15 min at 25°C. The hybridized probe was detected by using the Genius 3 (Boehringer Mannheim) chemiluminescence kit as per the manufacturer's instructions.

Southern blotting was performed to confirm positive PCR products. Amplified products were separated on a 2% SeaKem

GTG agarose (FMC, Rockland, Maine) gel and blotted onto a Hybond-N+ membrane by using a PosiBlot System (Stratagene) as per the manufacturer's instructions. Membranes were hybridized as previously described for the dot blots.

Isolated DNA amplified with the *uidA* PCR primers were blotted as described above and hybridized with the labeled oligonucleotide, UAR-900 (5'-ACGCGTGGTTACAGTCTT GCG-3') described by Bej et al. (1). Hybridization and detection conditions for the UAR-900 probe were the same as described above.

Bioassay. Bacterial isolates that were positive after amplification with the LT, SLT I, or SLT II primers and hybridized with internal probes were tested for toxin production in a bioassay by the California State Department of Health in a blind study. Mouse Y-1 adrenal cells and the human Vero cell line were used to test for toxin production by positive isolates. A random set of four toxin-negative *E. coli* isolates as well as one ATCC isolate positive for either SLT II or LT were used as controls in bioassays. The SLT-positive ATCC strains and the SLT-positive environmental isolate were tested for O157 antigen by using the Prolex (Ontario, Canada) latex agglutination kit.

Seeding of *E. coli* into ocean water and tap water. Three *E. coli* strains (ATCC 43886, 43889, and 43890) were added to filter-sterilized ocean water or tap water treated with thiosulfate to neutralize chlorine residuals. All three strains were added at concentrations of 2×10^6 cells per strain per ml. Tenfold dilutions were made in sterile deionized water. Five milliliters of each dilution was filtered through a 25-mm Teflon FHLP filter (Millipore, Bedford, Mass.). The filter was placed in 100 μl of sterile deionized water and vortexed for 20 s to remove the cells from the filter. The filter was removed, and the remaining wash was subjected to five freeze-thaw cycles consisting of 5 min in a dry ice-ethanol bath and 5 min in a 65°C water bath. The lysate was added to 2 ml of sterile water in a Centricon 100 microconcentrator (Amicon, Beverly, Mass.) and concentrated to 100 μl according to the manufacturer's directions. The concentrated samples were stored at 4°C. The PCR parameters were modified from the previous description in order to adjust for reduced template concentrations. Primer concentrations of LT and SLT II were changed to 0.8 and 0.1 μM, respectively, and PCR was extended to 40 cycles. Primer concentration of SLT I remained at 0.3 μM. Positive controls included genomic DNA from individual isolates or a mixture of DNA from all three isolates. Amplified products were further analyzed by Southern blotting and hybridization as described above.

RESULTS

***E. coli* control strains.** PCR and hybridization results indicate that ATCC 35401 and ATCC 43886 were positive for the LT homologous fragment; ATCC strains 43890, 43894, and 43895 were positive for the SLT I homologous sequence; and ATCC strains 43889, 43894, and 43895 were positive for a SLT II homologous PCR fragment (Fig. 1). Non-PCR-amplified DNA templates for ATCC strains and a random selection of environmental isolates were negative for internal probe hybridization (data not shown). All ATCC SLT I and SLT II strains were O157 positive, as stated in the ATCC catalog and confirmed by using the *E. coli* O157 latex agglutination test.

***E. coli* environmental isolates.** In total, 377 environmental *E. coli* isolates were amplified and screened for the presence of LT, SLT I, and SLT II homologous sequences by using PCR and internal oligonucleotide probe hybridizations. Seventeen non-*E. coli* environmental isolates were screened as negative controls to ensure PCR primer and probe specificity, and none hybridized with the oligonucleotide probes. Hybridization results of the California *E. coli* isolates indicate that one hybridized with the LT probe. The North Carolina estuary *E. coli* isolates showed that one hybridized with the SLT I probe after PCR amplification (Table 1).

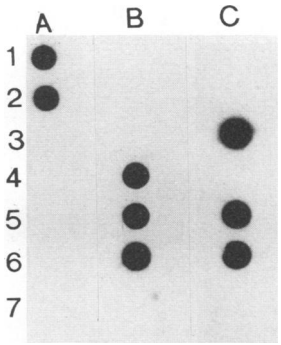

FIG. 1. Triplex PCR products of ATCC control strains dot blotted and hybridized with internal probes. Bacterial strains were amplified for LT, SLT I, and SLT II homologous sequences. The bacterial strains are as follows: Row 1, ATCC 35401 (LT and ST); row 2, ATCC 43886 (LT); row 3, ATCC 43889 (SLT II); row 4, ATCC 43890 (SLT I); row 5, ATCC 43894 (SLT I and SLT II); row 6, ATCC 43895 (SLT I and SLT II); and row 7, ATCC 43896 (ST). PCR products were blotted and hybridized with LT (lane A), SLT I (lane B), and SLT II (lane C), which hybridized with LT, SLT I, and SLT II homologous sequences, respectively.

Southern blotting. The presumptive LT- and SLT I-positive *E. coli* strains were tested by Southern analysis for confirmation. The expected size of the LT-amplified fragment was 258 bp, while a smaller PCR product of 130 bp was the expected size for the SLT I-amplified fragment. The correct fragment size was observed for both LT and SLT I amplifications. The bands were detected with the internal probe after Southern blotting. In the LT-containing strain, however, an additional band of approximately 172 bp was observed on the autoradiograph but was not visible on the ethidium bromide-stained agarose gel (Fig. 2).

***uidA* gene.** All Southern California *E. coli* isolates (249 of 249) were tested for the presence of the *uidA* gene fragment. Three DNA extracts from non-*E. coli* isolates were included as negative controls. DNA samples that were used for triplex PCR were amplified for a region of the *uidA* gene to confirm that the DNA extracts did not inhibit the PCR. All (100%) of the PCR products from the Southern California *E. coli* extracts hybridized with the UAR-900 oligonucleotide (data not shown). The non-*E. coli* isolates were all negative for the *uidA* gene.

TABLE 1. Analysis of *E. coli* strains isolated from California and North Carolina coastal water for toxigenicity by using PCR with subsequent DNA hybridization

Source	*n*	No. positive (%)	Toxin type
California			
Surfzone	186	0 (0)	NA[a]
Outfall	63	1 (1.6)	LT
North Carolina			
Closed estuary	117	1 (0.8)	SLT I
Oyster	11	0 (0)	NA

[a] NA, not applicable.

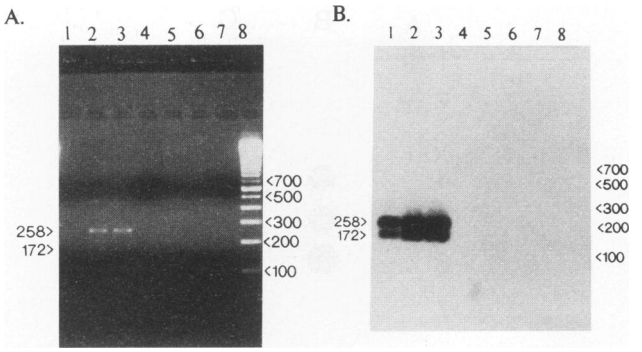

FIG. 2. Agarose gel electrophoresis and Southern hybridization of PCR products. The presumptive LT-positive *E. coli* and six ATCC *E. coli* control strains were amplified for LT. (A) PCR products separated on a 2% agarose gel; (B) PCR products blotted and hybridized with LT for the detection of amplified LT homologous sequences. Lanes: 1, presumptive LT-positive *E. coli* isolate; 2, ATCC 35401 (LT and ST); 3, ATCC 43886 (LT); 4, ATCC 43889 (SLT II); 5, ATCC 43890 (SLT I); 6, ATCC 43895 (SLT I and SLT II); 7, ATCC 43896 (ST); and 8, 100-bp molecular weight marker.

Seeding experiments. Gel electrophoresis of amplified products from the seeding experiment showed that three target fragments (346, 130, and 258 bp) were amplified from DNA extracts of a mixture of three *E. coli* strains containing the SLT II, SLT I, and LT genes, respectively (Fig. 3A). Other samples contained DNA extracts from individual isolates. Southern analysis confirmed that the three *E. coli* strains seeded in ocean and tap water could be detected in a single amplification procedure (Fig. 3B).

Environmental samples from tap water or ocean water containing cells of all three strains were subjected to DNA extraction and triplex PCR. All PCR products were electrophoresed in triplicate, and each replicate was detected with a single probe (Fig. 3, lanes 1 to 4, SLT II; lanes 11 to 14, SLT I; and lanes 21 to 24, LT). Additionally, triplex PCR was performed with previously extracted DNA in a mixture of three strains as a positive control (Fig. 3, lanes 8, 18, and 28). The triplex results on the seeded samples and DNA mixture were confirmed by using DNA from each strain as the sole template. No cross-reactivity was found between each strain by using the other probes (Fig. 3, lanes 5 and 7, lanes 15 and 16, and lanes 26 and 27).

Bioassay. Both positive environmental isolates produced cytotoxic effects in cell culture. The LT-positive isolate was cytotoxic on the mouse Y-1 adrenal cell line, while the SLT I strain was cytotoxic on Vero cells. Strains not positive for toxin fragments were negative on these cell lines.

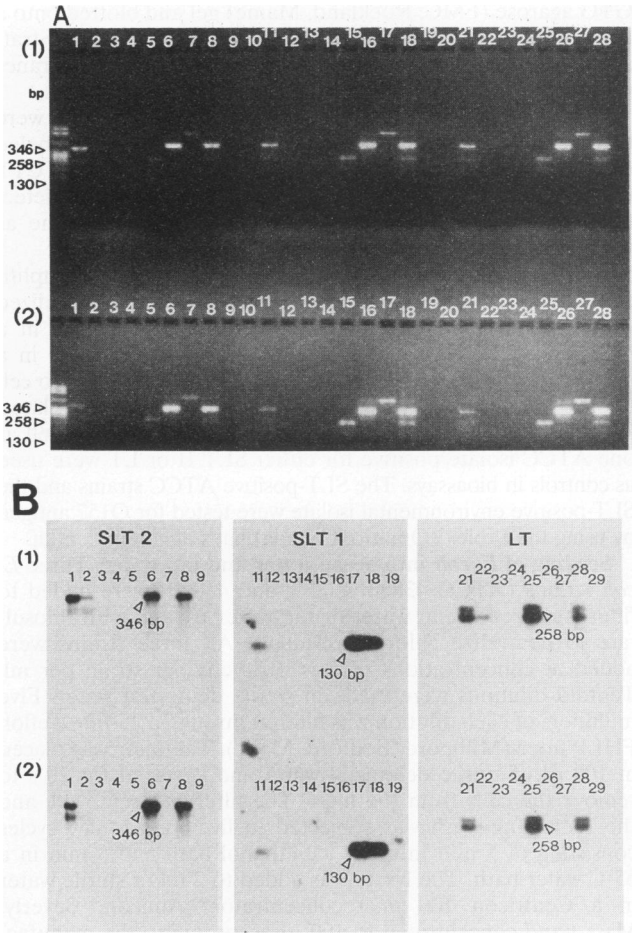

FIG. 3. Triplex PCR products amplified from DNA extracted from tap water (1) and ocean water (2) seeded with control strains *E. coli* ATCC 43886, 43889, and 43890. (A) Digitized picture of the amplified product on an ethidium bromide-stained agarose gel after gel electrophoresis; (B) Southern analysis of the agarose gel in panel A hybridized with SLT 2 (lanes 1 to 9), SLT 1 (lanes 11 to 19), and LT (lanes 21 to 29). PCR mixtures containing all three primer sets and total DNA equivalent to 10^5 to 10^2 organisms per strain in triplicate are in lanes 1 to 4, 11 to 14, and 21 to 24. Lanes 5, 15, and 25, amplified DNA (258 bp) from ATCC 43886 (LT); lanes 6, 16, and 26, amplified DNA (346 bp) from ATCC 43889 (SLT 2); lanes 7, 17, and 27, amplified DNA (130 bp) from ATCC 43890 (SLT 1); lanes 8, 18, and 28 strains ATCC 43886, 43889, and 43890 together; lanes 9, 19, and 29, negative controls containing no template. Molecular weight markers are shown in the leftmost lane of panel A.

DISCUSSION

The development of molecular methodologies to detect pathogenic microorganisms in clinical and environmental samples has led to improved patient diagnosis and more precise determination of the public health risk associated with environmental exposures. This study reports on the development of a triplex PCR methodology that allows for the simultaneous detection of three different types of *E. coli* associated with human disease. The use of three sets of primers in a single PCR mixture allowed for successful amplification of SLT I, SLT II, and LT homologous sequences in ATCC *E. coli* control strains. Southern blotting and internal probes were used to confirm the target PCR products. The PCR triplex method was then successfully used to screen environmental isolates for SLT I, SLT II, and LT homologous sequences.

Testing 377 environmental isolates for the presence of toxigenic *E. coli* by using the triplex PCR and subsequent DNA hybridization indicated that one Southern California deepwater *E. coli* isolate had an LT homologous sequence and none was positive for SLT I or SLT II gene fragments. The LT-positive isolate may have originated from the treated sewage effluent discharged offshore. However, since only one positive isolate was found, there may be a low incidence of toxigenic *E. coli* in treated sewage effluent. Studies to determine the percentage of toxigenic *E. coli* in treated sewage effluent at the treatment plant are currently under way. Southern California offshore coastal waters were negative for toxi-

genic *E. coli*, suggesting that the treated effluent was not reaching nearshore bathing areas. Toxigenic *E. coli* strains were also present in low numbers in North Carolina waters that were closed for shellfish harvesting. Only one isolate was found to be positive with the SLT I primers, and none was positive with the SLT II or LT primers. Our results suggest that toxigenic *E. coli* occurs infrequently in coastal waters. The lack of toxigenic *E. coli* in environmental samples has been reported previously (17). Our study concurs with this finding and indicates a low public health risk from toxigenic *E. coli* in coastal waters.

One problem encountered during the development of this assay was the appearance of a double fragment (172 and 258 bp) on LT amplifications performed on ATCC strains. Subsequent Southern analysis confirmed that the hybridization signal was not caused by contamination and that one of the amplified target bands was the expected size for the LT target fragment—258 bp. The 172-bp PCR fragment observed on the Southern blot is believed to be a secondary PCR fragment. Additionally, although not detected in extracted DNA samples, a secondary band was observed when whole cells were amplified with SLT II primers. Similar fragments were reported on by Pollard et al. (12) with the SLT II primers; however, DNA hybridization or sequencing was not performed to confirm that the unknown fragments were not SLT II related. The fact that SLT toxin production has been demonstrated in *Escherichia*, *Shigella*, *Salmonella*, *Vibrio*, and *Campylobacter* species (10) led Jackson et al. (7) to believe that SLT I and SLT II are members of a larger gene family. This may explain the smaller PCR product detected with the internal SLT II oligonucleotide probe from the seeded ocean water (Fig. 3B2, lane 1). Consequently, the unidentified fragments may represent SLT or SLT-like sequences which may or may not be associated with virulence.

In conclusion, molecular technologies offer great promise in monitoring the environment for pathogenic microorganisms and the early detection of clinical disease. Classical bioassays clearly identify organisms that are phenotypically pathogenic, but bioassays are not practical for screening large numbers of isolates or for direct detection of toxigenic *E. coli*. Thus, they have limited use in environmental or clinical detection. This study used a triplex PCR method to rapidly screen *E. coli* isolated from the environment for LT, SLT I, and SLT II homologous sequences. Confirmation of the PCR findings with traditional bioassay techniques lends further credibility to the technology. The application of this procedure to water samples may prove to be a valuable tool for rapidly screening large samples for the presence of potentially pathogenic *E. coli*.

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