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Optimization of Rapid Detection of *Escherichia coli* O157:H7 and *Listeria monocytogenes* by PCR and Application to Field Test

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ABSTRACT

For rapid detection of *Escherichia coli* O157:H7 and *Listeria monocytogenes*, simple methods for sample preparation and PCR were established and applied to a field test. To improve specificity, primer sets LP43-LP44 and C(+)-D(−) were selected for *E. coli* O157:H7 and *L. monocytogenes*, respectively. Through centrifugation and partial heat treatment after enrichment, *E. coli* O157:H7 and *L. monocytogenes* were detected at 1 initial CFU without genomic DNA extraction in the culture and with artificially inoculated food samples including milk, chicken, ham, and pork. Based on the optimized PCR method, a feasibility test was carried out using randomly collected field samples. To remove false positives and false negatives, a PCR method using several primer sets, including the optimized primer set, and a standard culture method were used. With the PCR detection and standard culture methods, two pork samples were positive for *L. monocytogenes* after enrichment, indications that the PCR assay could be effectively used for rapid, sensitive, and species-specific detection of foodborne pathogens.

Recently, microbial food safety issues have emerged as a major public health concern worldwide *(13).* Therefore, methods for effective detection of foodborne pathogens such as *Escherichia coli* O157:H7 and *Listeria monocytogenes* are required for the prevention and control of microbial contamination *(13).* Recognized in 1982, enterohemorrhagic *E. coli* strains cause severe and bloody diarrhea, hemolytic uremic syndrome, and in some cases hemorrhagic colitis *(15, 16, 22). E. coli* O157:H7 is a member of the enterohemorrhagic *E. coli* group, and most *E. coli* O157:H7 infections have been associated with the consumption of contaminated ground beef, milk, water, and apple juice products that have been improperly handled, stored, or cooked *(1, 8, 9).* Although *L. monocytogenes* has been recognized as a human pathogen for >70 years, it was classified as a foodborne pathogen only two decades ago *(12).* This organism causes listeriosis in pregnant women, cancer patients, the elderly, and immunocompromised patients *(5, 12).* Food products such as milk, cheese, meat, and ready-to-eat foods have been reported as target contamination sources associated with human listeriosis *(12, 14, 23).*

For decades, various molecular diagnostic techniques have been used to monitor foodborne pathogens for public health protection. PCR-based detection methods are powerful tools because of their very high specificity and simplicity *(2, 5, 24, 25).* Genes targeted for specific detection by PCR are related to virulence factors of the pathogen, such as genes for O-antigen biosynthesis (*rfb* gene), glucuronidase (*uidA* gene), verotoxins (Shiga-like toxins, stx_1 and stx_2 genes), and proteins related to attaching and effacing (*eaeA* gene) *(7, 11, 19)* for *E. coli* O157:H7 and genes for protein p60 (*iap* gene) and listeriolysin O (*hlyA* gene) *(3, 20, 21)* for *L. monocytogenes.* In several studies, *iap*-derived primers have been applied for the specific identification of *L. monocytogenes (4, 6, 10).* In addition, a simple method for simultaneous specific identification and differentiation of *L. monocytogenes* based on the PCR method was established using a hypervariable internal *iap* gene fragment *(3).*

Separation of the target pathogen from food matrices and preparation of template DNA are important prerequisites for PCR amplification. Because of the presence of inhibitors influencing the effectiveness of PCR, efficient sample treatment methods, such as varying the bacterial concentration and removal of PCR inhibitors from food, have been developed to apply the PCR-based assays for detection of virulent foodborne pathogens *(17, 18).* Accordingly, several methods for removing PCR inhibitors and increasing the sensitivity of PCR detection have been suggested, including the use of centrifugation, detergents, lysozyme, a single enrichment medium, and antibody-coated magnetic beads *(2, 24, 25).* Commercially available kits for extraction of target pathogen genomic DNA from foods are best suited for these procedures. However, the use of commercial kits without any pretreatment process has some limiting factors, such as small amount of target organism, presence of PCR inhibitors, and low benefits in terms of time and cost. Thus, we have worked hard to establish conditions for simplifying sample preparation and increasing the sensitivity and specificity of these procedures while decreasing the amount of time and labor needed. In this study, attempts were made to optimize a simple step for the preparation of extraction-free PCR template under reliable PCR conditions

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TABLE 1. *Oligonucleotide primers used in this study*

Primer	Sequences (5′→3′)	Target gene	PCR product (bp)	References
PF8	CGTGATGATGTTGAGTTG	*rfb* gene	420	*19*
PR8	AGATTGGTTGGCATTACTG			
PT2	GCGAAAACTGTGGAATTGGG	*uidA* gene	252	*7*
PT3	TGATGCTCCATCACTTCCTG			
LP30	CAGTTAATGTGGTGGCGAAGG	stx_1 gene	348	*7*
LP31	CACCAGACAATGTAACCGCTG			
LP43	ATCCTATTCCCGGGAGTTACG	stx_2 gene	584	*7*
LP44	GCGTCATCGTATACACAGGAGC			
AE19	CAGGTCGTCGTGTCTGCTAAA	*eaeA* gene	1,087	*11*
AE20	TCAGCGTGGTTGGATCAACCT			
C(+)	TTACGAATTAAAAAGGAGCG	Downstream of *hlyA* gene *iap* gene	161	*20, 21*
D(−)	TTAAATCAGCAGGGGTCTTT			
MonoA	CAAACTGCTAACACAGCTACT		660	*3*
Lis1B	TTATACGCGACCGAAGCCAAC			

using a model food, and the applicability of this process was tested on field samples to monitor microbial hazards in markets and industries.

MATERIALS AND METHODS

Bacterial strains and culture conditions. *E. coli* O157:H7 ATCC 43894, *L. monocytogenes* ATCC 19111, and *Staphylococcus aureus* ATCC 14458 were purchased from the American Type Culture Collection (Rockville, Md.). *E. coli* KCTC 1039, *E. coli* KCTC 1041, and *E. coli* KCTC 1114 were purchased from the Korean Collection for Type Cultures (Seoul, Korea). *Salmonella* Enteritidis P1 was isolated in our laboratory. *E. coli* O157:H7 was aerobically cultured in modified *E. coli* broth (mEC broth; Merck, Darmstadt, Germany) at 37°C for 18 h. For the selective enumeration of *E. coli* O157:H7, cefixime tellurite–sorbitol MacConkey (CT-SMAC) agar (Merck) was used. *L. monocytogenes* was aerobically cultured in *Listeria* enrichment broth (LEB; Merck) at 37°C for 18 h. For the selective enumeration of *L. monocytogenes*, Oxford *Listeria* selective agar with supplement (LSA; Merck) was used. *E. coli* KCTC 1039, *E. coli* KCTC 1041, and *E. coli* KCTC 1114 were cultured aerobically in Luria-Bertani broth (10 g tryptone, 5 g yeast extract, 5 g NaCl per liter) at 37°C for 18 h. *Salmonella* Enteritidis P1 and *S. aureus* were cultured aerobically in nutrient broth (Merck) at 37°C for 18 h.

PCR primers. Sequences and references of the oligonucleotide primers synthesized by a private biotechnology company (Bioneer, Cheongwon, Korea) are given in Table 1.

PCR template preparation from pure culture. Culture aliquots of *E. coli* O157:H7 and *L. monocytogenes* were centrifuged at 13,000 rpm for 5 min, and the supernatant was discarded. The cell pellet was washed twice with 1 ml of distilled deionized water and resuspended to a 0.1-ml volume with distilled deionized water. Two microliters of the cell suspension was used directly for the PCR amplification. For comparison of sensitivities in terms of template types, whole cell, cell lysate obtained after heating (98°C, 10 min), and genomic DNA isolated with a commercial kit (AquaPure genomic DNA isolation kit, Bio-Rad, Hercules, Calif.) were used as templates.

Inoculation of *E. coli* O157:H7 and *L. monocytogenes* into model foods. To evaluate the effect of food matrix on template preparation, *E. coli* O157:H7 and *L. monocytogenes* were artificially inoculated into whole milk, raw chicken meat, ham, and cheese, which were freshly purchased from a local market. Nine milliliters of milk was placed into a sterile test tube, and 25 g of each minced sample was added into a sterile stomacher filter bag (Seward, Thetford, Norfolk, UK) with 100 ml of enrichment broth (mEC broth or LEB) or 0.1% peptone water. After stomaching at 200 rpm for 1 min with a Stomacher 400 circulator (Seward), 9 ml of each filtrate was distributed into sterile test tubes. *E. coli* O157:H7 and *L. monocytogenes* were grown in mEC broth and LEB, respectively, for 18 h at 37°C, inoculated into each sample, and diluted to final concentrations of 0 to 10^5 CFU/ml (g) of the sample. In addition, for comparison with sensitivities of model foods in terms of template types, cell lysate obtained after heating (98°C, 10 min) and genomic DNA isolated with commercial kit (Bio-Rad) were used as PCR templates. The artificially inoculated samples were placed in a shaking incubator set at 37°C for enrichment, and sampling was carried out after 0, 4, 8, and 24 h of incubation. To determine the numbers of *E. coli* O157:H7 and *L. monocytogenes* during enrichment, CT-SMAC agar and LSA, respectively, were used. For PCR template preparation, 1 ml of the subsample was centrifuged at 13,000 rpm for 5 min, and the supernatant was decanted. The pellet was washed twice with distilled deionized water, resuspended in 0.1 ml of distilled deionized water, and heated at 98°C for 10 min. Two microliters of the cell lysate was directly used for PCR amplification (Fig. 1).

Field test. Ten raw pork, eight raw chicken, 10 traditional Korean sausage (*Sundae*) samples, and 10 steamed pork legs (*Jockbal*) were purchased from different retail shops and local open markets in Seongnam and Seoul, Korea. The collected samples were immediately placed in insulated shipping coolers containing frozen gel packs and examined in the laboratory according to standard cultural methods. The following PCR method also was used: 100 μl of the enriched sample homogenate, before pretreatment for PCR, was spread on selective media (CT-SMAC agar and LSA) for detection of *E. coli* O157:H7 and *L. monocytogenes*, respectively, incubated, and examined.

PCR assays. PCR was performed using 2 μl of template in a 20-μl volume of the PCR PreMix (Bioneer). The PCR mixture consisted of 1 U thermostable DNA polymerase, 250 μM each dNTP, 50 mM Tris-HCl (pH 8.3), 40 mM KCl, and 1.5 mM $MgCl_2$. PCR was carried out in a gene cycler (model 10167, Bio-Rad, Tokyo, Japan). The optimized cycle program of denaturation, annealing, and extension temperatures was as follows: 1 cycle of 2 min at 94°C; 35 cycles of 30 s at 94°C, 30 s at 55°C, and 1 min

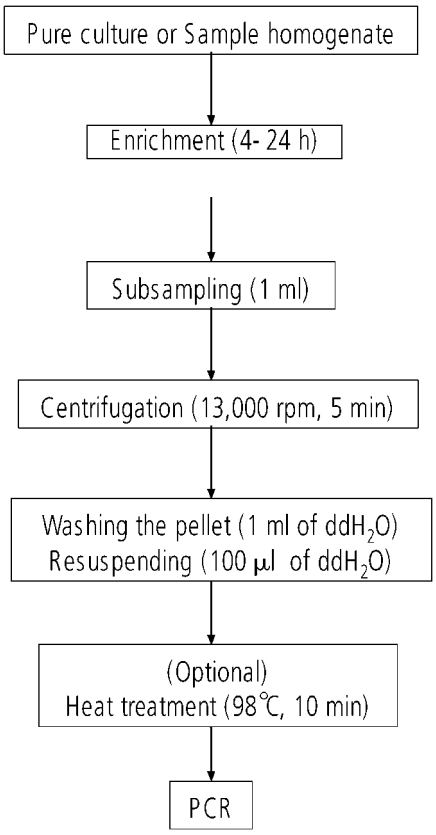

FIGURE 1. *Flow diagram of experimental protocols for PCR template preparation.*

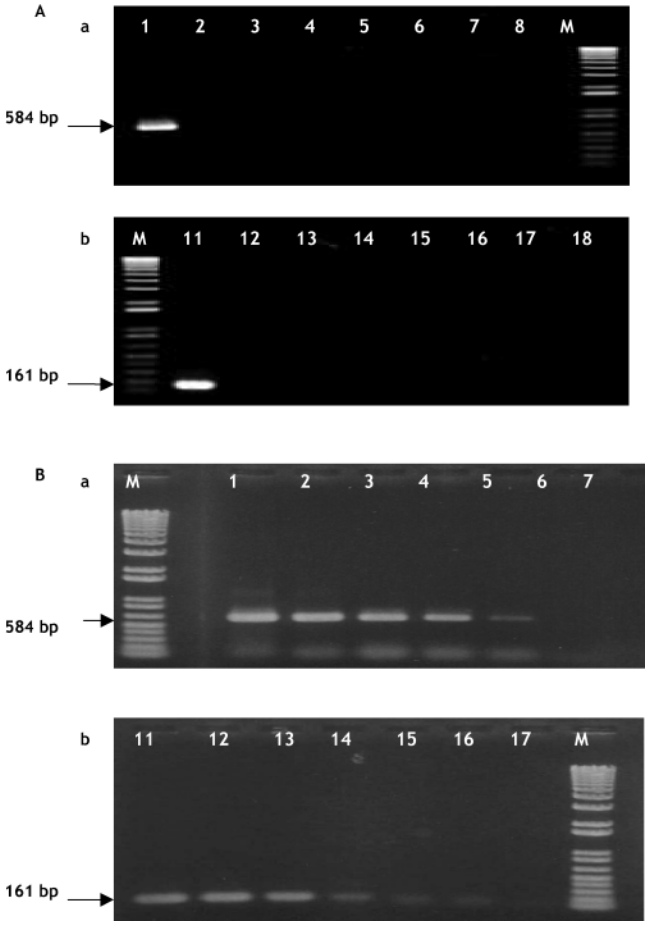

FIGURE 2. *Specificity (A) and sensitivity (B) tests for* E. coli *O157:H7 and* L. monocytogenes *using the LP43-LP44 primer set for the detection of* E. coli *O157:H7 (a) and the C(+)-D(−) primer set for the detection of* L. monocytogenes *(b). (A) Lane 1,* E. coli *O157:H7 ATCC 43894; lane 2,* E. coli *KCTC 1039; lane 3,* E. coli *KCTC 1041; lane 4,* E. coli *KCTC 1114; lane 5,* L. monocytogenes *ATCC 19111; lane 6,* Salmonella *Enteritidis P1; lane 7,* Staphylococcus aureus *ATCC 14458; lane 8, negative control (no template). M, 1-kb plus ladder (GIBCO BRL, Invitrogen, Carlsbad, Calif.). Lane 11,* L. monocytogenes *ATCC 19111; lane 12,* L. grayi *KCTC 3581; lane 13,* L. innocua *KCTC 3586; lane 14,* E. coli *O157:H7 ATCC 43894; lane 15,* E. coli *KCTC 1039; lane 16,* Salmonella *Enteritidis P1; lane 17,* S. aureus *ATCC 14458; lane 18, negative control (no template). M, 1-kb plus ladder (GIBCO BRL). (B) Lane 1, 2.1 × 10^5; lane 2, 2.1 × 10^4; lane 3, 2.1 × 10^3; lane 4, 2.1 × 10^2; lane 5, 21; lane 6, 2.1 CFU. Lane 7, negative control (no template). M, 1-kb plus ladder (GIBCO BRL). Lane 11, 1.4 × 10^6; lane 12, 1.4 × 10^5; lane 13, 1.4 × 10^4; lane 14, 1.4 × 10^3; lane 15, 1.4 × 10^2; lane 16, 14 CFU. Lane 17, negative control (no template). M, 1-kb plus ladder (GIBCO BRL).*

at 72°C; and 1 cycle of 5 min at 72°C. All PCR experiments were performed in triplicate. The PCR products were analyzed using 1.5% agarose gel electrophoresis and ethidium bromide staining. The gel was visualized and photographed under UV light.

RESULTS AND DISCUSSION

Specificity and sensitivity for selected primer set. To select the primer set most specific for *E. coli* O157:H7, PT2-PT3, PF8-PR8, LP30-LP31, LP43-LP44, and AE19-AE20 primer sets based on *uidA*, *rfb*, *stx*$_1$, *stx*$_2$, and *eaeA* genes, respectively, were examined. After PCR amplification, a 584-bp band amplified with the LP43-LP44 primer set was most specifically detected, whereas several nonspecific bands were amplified with other primer sets (data not shown). To screen *L. monocytogenes*–specific primer sets, C(+)-D(−) and MonoA-Lis1B sets based on the downstream *hlyA* and *iap* genes, respectively, were evaluated. The agarose gel revealed a clear 161-bp band amplified with C(+)-D(−) (data not shown). PCRs were performed to test the specificity of the selected primer sets, LP43-LP44 and C(+)-D(−), using the following as reference species: LP43-LP44 set: *E. coli* O157:H7 ATCC 43894, *E. coli* KCTC 1039, *E. coli* KCTC 1041, *E. coli* KCTC 1114, *L. monocytogenes* ATCC 19111, *S. aureus* ATCC 14458, and *Salmonella* Enteritidis P1; C(+)-D(−) set: *L. monocytogenes* ATCC 19111, *L. grayi* KCTC 3581, *L. innocua* KCTC 3586, *E. coli* O157:H7 ATCC 43894, *E. coli* KCTC 1039, *S. aureus* ATCC 14458, and *Salmonella* Enteritidis P1. The primer sets generated specific bands of 584 and 161 bp for *E. coli* O157:H7 ATCC 43894 and *L. monocytogenes* ATCC 19111, respectively (Fig. 2A, lanes 1 and 11), whereas no bands were observed for the other reference species (Fig. 2A, lanes 2 through 7 and 12 through 17) and the negative control (Fig. 2A, lanes 8 and 18). These results indicate that the primer sets LP43-LP44 and C(+)-D(−) were appropriate for the species-specific detection of *E. coli* O157:H7 and *L. monocytogenes*, respectively.

In addition, detection sensitivities of LP43-LP44 and

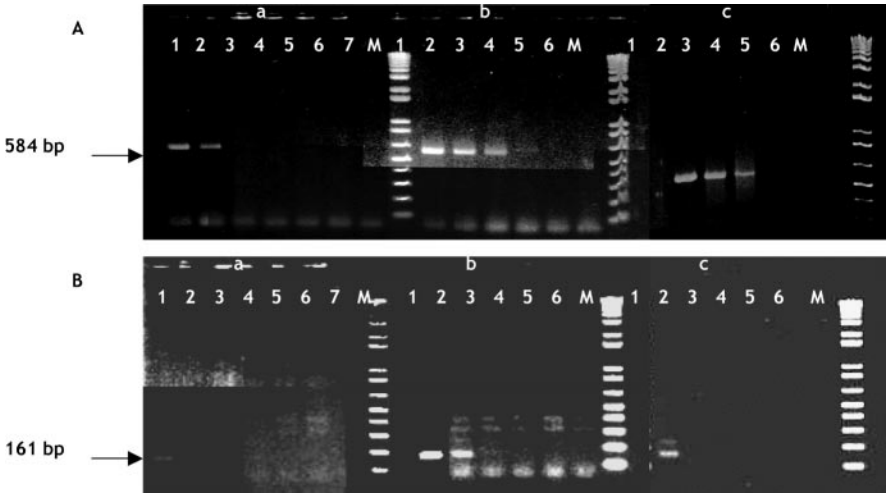

FIGURE 3. *Comparison of sensitivities for* E. coli *O157:H7 (A) and* L. monocytogenes *(B) according to template types in artificially inoculated milk using the LP43-LP44 and C(+)-D(−) primer sets. Templates used were whole cell (a), heat-treated cell lysate (b), and genomic DNA isolate (c). (A) Lane 1, 2.1 × 10^6; lane 2, 2.1 × 10^5; lane 3, 2.1 × 10^4; lane 4, 2.1 × 10^3; lane 5, 2.1 × 10^2 CFU/ml of milk. Lane 6, no spiked sample; lane 7, negative control (no template). M, 1-kb plus ladder (GIBCO BRL). (B) Lane 1, 7.2 × 10^6; lane 2, 7.2 × 10^5; lane 3, 7.2 × 10^4; lane 4, 7.2 × 10^3; lane 5, 7.2 × 10^2 CFU/ml of milk. Lane 6, no spiked sample; lane 7, negative control (no template). M, 1-kb plus ladder (GIBCO BRL).*

C(+)-D(−), which were evaluated using whole cell cultures of *E. coli* O157:H7 ATCC 43894 and *L. monocytogenes* ATCC 19111, were 10 and 1,000 cells, respectively, in the pure cultures (Fig. 2B). Based on these results, LP43-LP44 and C(+)-D(−) primers was selected for sensitive and species-specific detection of *E. coli* O157:H7 and *L. monocytogenes*, respectively.

Effect of food matrix on detection of *E. coli* O157:H7 and *L. monocytogenes*. Development of simple template preparation techniques is necessary prior to the application of the PCR method to field samples for detecting foodborne pathogens. To simplify the procedure of sample preparation and to improve the detection of target pathogens, centrifugation and heat treatment, individually or in combination, were used for the removal of PCR inhibitors from food matrices.

After centrifugation of whole milk artificially contaminated with *E. coli* O157:H7 and *L. monocytogenes* (10^6 to 10^2 CFU/ml) at 13,000 rpm for 5 min without further steps, the expected DNA fragments were amplified and specifically detected on the agarose gel, indicating that the milk matrix had no PCR amplification inhibitors. However, when used in combination with heat treatment, the detection sensitivity increased, showing a 1-log reduction in both *E. coli* O157:H7 and *L. monocytogenes* compared with the whole cell results (Fig. 3A and 3B). To examine the effects of food matrix, including milk, raw chicken meat, ham, and cheese, on the sensitivity of the PCR, populations of *E. coli* O157:H7 and *L. monocytogenes* after enrichment at 37°C over time were counted on CT-SMAC agar and LSA, respectively, and the PCR was carried out as described above. Upon PCR amplification, *E. coli* O157:H7 was detected at 10^4 and 10^0 initial CFU/ml of milk before and after enrichment for 8 h, respectively (Table 2 and Fig. 4A, a and c). However, *L. monocytogenes* was detected at only 10^5 and 10^2 initial CFU/ml of milk before and after enrichment for 24 h, respectively (Table 2 and Fig. 4B, a and d). These results indicate that raw milk is a good medium for the cultivation of *E. coli* O157:H7 but not for *L. monocytogenes*. Nevertheless, this problem was overcome by cultivating *L. monocytogenes* in an enrichment broth, LEB (data not shown). Unfortunately, nonspecific bands were slightly amplified by the C(+)-D(−) primer set, an indication that complex food matrix and nonspecific food-derived contaminants existed in the food samples.

TABLE 2. *Detection limits for* E. coli *O157:H7 and* L. monocytogenes *in artifically inoculated food samples before and after enrichment*[a]

	Detection limit (initial CFU/ml or CFU/g)[c]							
	Before enrichment						After enrichment	
	Whole cell		Heat-treated cell lysate		Genomic DNA isolate		Heat-treated cell lysate	
Food sample[b]	Ec	Lm	Ec	Lm	Ec	Lm	Ec	Lm
Raw milk	≥10^5	10^6	≥10^4	10^5	≥10^4	10^6	≥1	1
Raw chicken meat	ND	ND	≥10^4	10^5	≥10^4	10^5	≥1	1
Ham	≥10^4	10^5	≥10^3	10^5	≥10^3	10^5	≥1	1
Cheese	ND	ND	ND	ND	≥10^3	10^5	≥1	1

[a] PCR was performed with 10 pmole primers using 2 μl of each preparative template in 20 μl PCR mixture.
[b] Each sample was artifically inoculated, 10^6 to 10^2 CFU/ml of milk; 10^6 to 10^3 CFU/g of raw chicken meat; 10^5 to 10^3 CFU/g of ham; 10^5 to 10^3 CFU/g of cheese sample.
[c] *E. coli* O157:H7 (Ec) and *L. monocytogenes* (Lm) were enriched for 8 h at 37°C and 24 h at 37°C, respectively. ND, no detection.

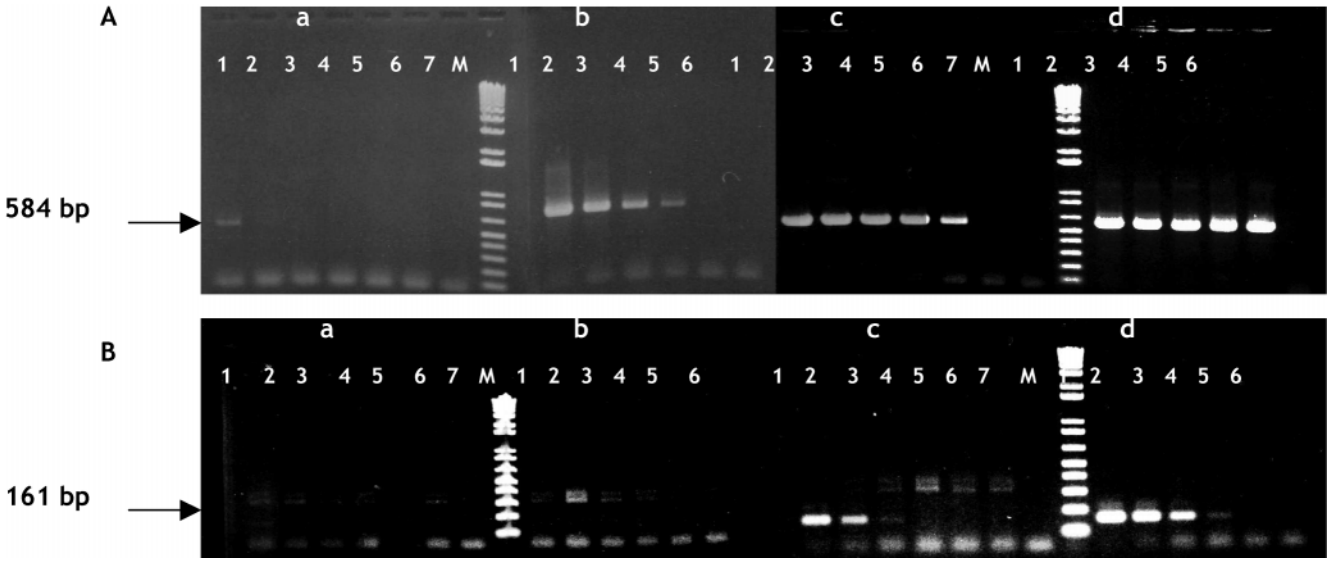

FIGURE 4. *Sensitivities of LP43-LP44 and C(+)-D(−) primer sets in detecting* E. coli *O157:H7 (A) and* L. monocytogenes *(B), repectively, from artificially inoculated milk samples during enrichment, which was performed using raw milk for 0 h (a), 4 h (b), 8 h (c), and 24 h (d). (A) Lane 1, 7.5 × 10⁴; lane 2, 7.5 × 10³; lane 3, 7.5 × 10²; lane 4, 75; lane 5, 7.5 initial CFU/ml of milk. Lane 6, no spiked sample; lane 7, negative control (no template). M, 1-kb plus ladder (GIBCO BRL). (B) Lane 1, 1.5 × 10⁵; 1ane 2, 1.5 × 10⁴; 1ane 3, 1.5 × 10³; 1ane 4, 1.5 × 10²; 1ane 5, 15 initial CFU/ml of milk. Lane 6, no spiked sample; lane 7, negative control (no template). M, 1-kb plus ladder (GIBCO BRL).*

Solid samples of raw chicken meat, ham, and cheese were stomached and enriched at 37°C in mEC broth or LEB. To investigate the efficiency of PCR detection in different sample matrices, three template types, whole cell, heat-treated cell lysate, and genomic DNA isolate, were prepared and examined. In the chicken meat sample, both *E. coli* O157:H7 and *L. monocytogenes* were not detected at 10^6 CFU/g without heat treatment (Fig. 5A, a). However, after heat treatment at 98°C for 10 min, the detection limit increased to 10^4 CFU/g for both pathogens (Fig. 5A, c). In contrast, preparation of PCR template from the ham sample involved a simple matrix, an indication of little matrix effect on PCR results. *E. coli* O157:H7 and *L. monocytogenes* were detected at 10^3 and 10^5 CFU/g, respectively, when heat-treated cell lysate was used. Sensitivity of the lysate was as high as that of genomic DNA isolated using a commercial kit (Table 2). Cheese, however, has different matrix characteristics. Upon stomaching with enrichment broth, the cheese homogenate changed into an emulsion, thereby making the phase separation hard to achieve. Thus, only the genomic DNAs purified from the pathogens in cheese were amplified, and the detection limits were comparatively high, 10^3 and 10^5 CFU/g of cheese for *E. coli* O157:H7 and *L. monocytogenes*, respectively (Table 2), but were as low as 1 initial CFU/g with heat-treated cell lysate after enrichment (Fig. 5B). Sensitivities of LP43-LP44 and C(+)-D(−) primers for detecting *E. coli* O157:H7 and *L. monocytogenes* appear to depend on the template type (Table 2). Nevertheless, *E. coli* O157:H7 and *L. monocytogenes* were detected at 1 initial CFU/ml (g) of the sample after enrichment using an appropriately prepared PCR template, the heat-treated cell lysate (Table 2).

Application of simple detection method to field samples. Simple techniques developed for rapid PCR detection were tested using field samples. To remove the possibilities of false-positive and false-negative results, four primer sets, including LP43-LP44 for *E. coli* O157:H7, and two primer sets, including C(+)-D(−) for *L. monocytogenes*, were used. Standard culture methods were run parallel with PCR using selection media. Templates prepared by centrifugation and subsequent heat treatment after enrichment for 24 h were directly applied to PCR amplification with species-specific primer sets. In screening tests using PT2-PT3 primers for *E. coli* O157:H7 (data not shown) and C(+)-D(−) primers for *L. monocytogenes* (Fig. 6A, a [lane 2] and 6A, b [lane 2]), positive bands were amplified in several food samples. However, in confirming tests using PF8-PR8, LP30-LP31, and LP43–44 for *E. coli* O157:H7 and MonoA-Lis1B (Fig. 6B) for *L. monocytogenes*, no *E. coli* O157:H7–specific bands were amplified in any sample, whereas bands specific for *L. monocytogenes* were amplified in two raw pork samples and its presence was confirmed through standard methods using selective media (data not shown). These results support the validity of using the PCR method for the detection of foodborne *E. coli* O157:H7 and *L. monocytogenes* in field samples.

Future work on molecular diagnostic procedures should include the development of rapid and reliable methods for detection of low levels of virulent microorganisms from a variety of samples. The appropriately developed methods can be ideally applied to routine monitoring of virulent microorganisms in food and food processing environments for disease control and protection of human health. Reduction in both time to results and the potential of false positives and false negatives could turn PCR-based methods into a powerful tool for sensitive and specific detection of foodborne pathogens. In this study, food samples that were liquid (milk), solid (raw pork meat, raw chicken

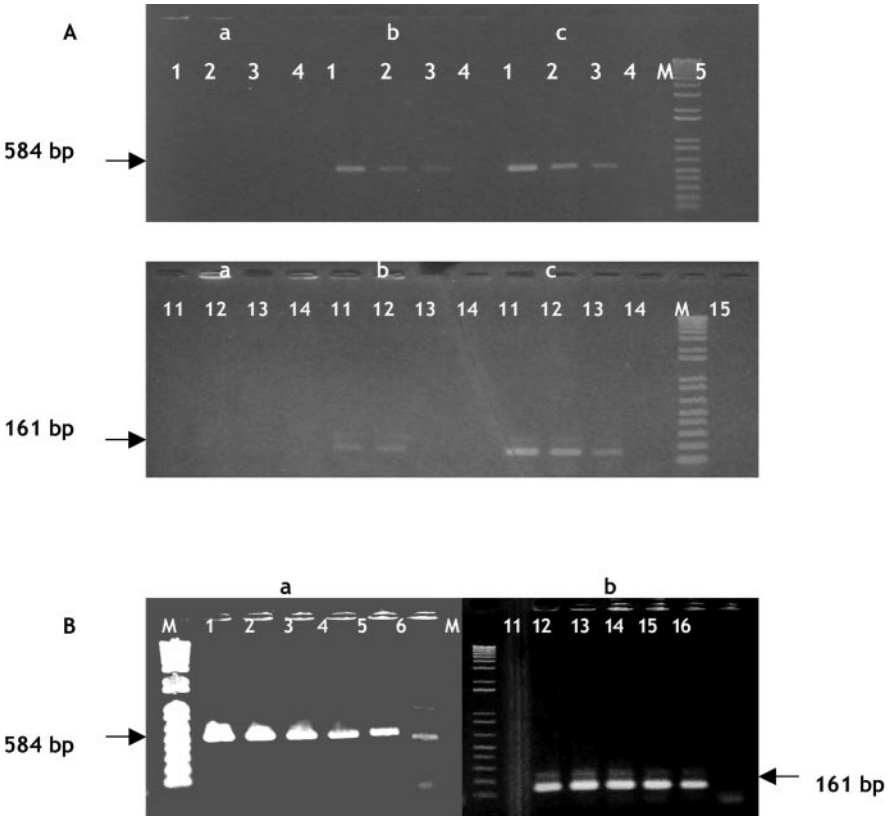

FIGURE 5. *Comparison of sensitivities according to template types in artificially inoculated chicken meat sample as determined by amplification with primers sets LP43-LP44 and C(+)-D(−) detecting* E. coli *O157:H7 (A, lanes 1 through 5) and* L. monocytogenes *(A, lanes 11 through 15), respectively, and sensitivities (B) of heat-treated cell lysates after enrichment for 24 h for detecting these pathogens. (A) Templates used were whole cell (a), heat-treated cell lysate (b), and genomic DNA isolate (c). Lane 1, 1.2 × 10^6; lane 2, 1.2 × 10^5; lane 3, 1.2 × 10^4; lane 4, 1.2 × 10^3 CFU/g of chicken meat sample. Lane 5, negative control (no template). M, 1-kb plus ladder (GIBCO BRL). Lane 11, 7.5 × 10^6; lane 12, 7.5 × 10^5; lane 13, 7.5 × 10^4; lane 14, 7.5 × 10^3 CFU/g of chicken meat sample. Lane 15, negative control (no template). M, 1-kb plus ladder (GIBCO BRL). (B) Pathogens tested were* E. coli *O157:H7 (a) and* L. monocytogenes *(b). Lane 1, 1.2 × 10^4; lane 2, 1.2 × 10^3; lane 3, 1.2 × 10^2; lane 4, 12; lane 5, 1.2 initial CFU/g of chicken meat sample. Lane 6, no spiked sample. M, 1-kb plus ladder (GIBCO BRL). Lane 11, 7.5 × 10^4; lane 12, 7.5 × 10^3; lane 13, 7.5 × 10^2; lane 14, 75; lane 15, 7.5 initial CFU/g of chicken meat sample. Lane 16, no spiked sample. M, 1-kb plus ladder (GIBCO BRL).*

meat, and ham), and semisolid (cheese) were homogenized. However, during this process the cheese sample became completely emulsified and could not be separated, even by centrifugation, which suggests that surface swabbing is a more appropriate method for recovering pathogens from semisolid dairy foods. The artificial contamination procedure posed limitations on evaluating the effect of food matrix on the efficacy of this PCR method. In this study, target

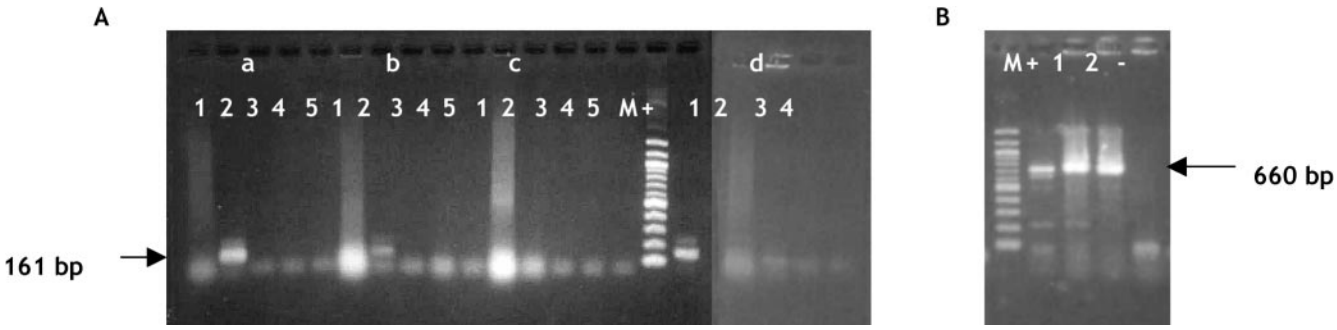

FIGURE 6. *Field sample testing using a simple technique for template preparation and PCR detection of* L. monocytogenes. *Screening and confirming tests were performed using the C(+)-D(−) primer set (A) and the MonoA-Lis1B primer set (B), respectively. (A) Samples were obtained from market A (a), supermarket B (b), traditional market C (c), and traditional market D (d). Lane 1, raw chicken meat; lane 2, raw pork; lane 3, steamed pork leg (*Jockbal*); lane 4, Korean traditional sausage (*Sundae*); lane 5, raw beef. +, Positive control (genomic DNA isolate); M, 100-bp ladder (New England BioLabs Inc., Beverly, Mass.). (B) Lane 1, market A raw pork; lane 2, supermarket B raw pork. −, negative control (no template); +, positive control (genomic DNA isolate); M, 100-bp ladder (New England BioLabs).*

pathogens were spiked into the homogenized food samples and recovered by centrifugation at 13,000 rpm for 10 min. For reliable and valid results, artificial inoculation should be carried out before homogenization, and the contaminated food samples should then be allowed to stand for several minutes at room temperature prior to further treatment. This PCR assay (without DNA extraction) for detection of *E. coli* O157:H7 and *L. monocytogenes* in meat and meat products was validated by the results presented here; *E. coli* O157:H7 and *L. monocytogenes* were detected at concentrations as low as 1 initial CFU/ml (g) after enrichment for 8 and 24 h, respectively.

Thus, the protocol developed in this study could have a significant impact on the rapid and easy detection of *E. coli* O157:H7 and *L. monocytogenes* in foods. This simple method will allow safe management of pathogen contamination of foods at a low cost.

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