Rapid Detection of *Escherichia coli* in Fresh Foods Using a Combination of Enrichment and PCR Analysis

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Abstract   The objective of this study was to determine the minimum enrichment time for different types of food matrix (pork, beef, and fresh-cut lettuce) in an effort to improve *Escherichia coli* detection efficiency. Fresh pork (20 g), beef (20 g), and fresh-cut lettuce (20 g) were inoculated at 1, 2, and 3 Log CFU/g of *Escherichia coli*. Samples were enriched in filter bags for 3 or 5 h at 44.5℃, depending on sample type. *E. coli* cell counts in the samples were enriched in *E. coli* (EC) broth at 3 or 5 h. One milliliter of the enriched culture medium was used for DNA extraction, and PCR assays were performed using primers specific for *uidA* gene. To detect *E. coli* (*uidA*) in the samples, a 3–4 Log CFU/mL cell concentration was required. However, *E. coli* was detected at 1 Log CFU/g in fresh pork, beef, and fresh-cut lettuce after 5, 5, and 3-h enrichment, respectively. In conclusion, 5-h enrichment for fresh meats and 3-h enrichment for fresh-cut lettuce in EC broth at 44.5℃, and PCR analysis using *uidA* gene-specific primers were appropriate to detect *E. coli* rapidly in food samples.

Keywords   fresh meat, fresh-cut lettuce, *Escherichia coli*, enrichment, PCR

Introduction

Food hygiene and safety are a major concern in the food industry, and microbiological safety is a particular problem. *Escherichia coli* can act as an indicator for the presence of other pathogenic bacteria, and it is detected easily in foods such as pork, beef, and chicken. Thus, *E. coli* detection in foods is one of the most useful hygienic criteria (Scheinberg et al., 2017; Seo et al., 2010; Simancas et al., 2016). However, at present the conventional method for *E. coli* detection requires several days (Feng et al., 2002; Stromberg et al., 2015; Wang and Salazar, 2016), especially in cases where *E. coli* concentrations are low. Enrichment is a commonly used method for bacterial isolation to

increase the cell counts of target bacteria above other background flora prior to identification (Gracias and McKillip, 2004). According to FDA-BAM (U.S. Food and Drug Administration-bacteriological analytical manual) and other reports, *E. coli* can be enriched with *E. coli* (EC) broth or modified tryptic soy broth (mTSB); however, the enrichment methods are time-consuming (Feng et al., 2002; Stromberg et al., 2015).

Polymerase chain reaction (PCR), using primers against the *uidA* gene that encodes beta-D-glucuronidase can be used to identify *E. coli* accurately (Molina et al., 2015). PCR detection method has been used to identify a colony on an agar plate, which was formed by plating at least 24-h enriched broth. However, applying PCR detection method directly to the enriched samples has not been evaluated yet. In addition, there is an issue of specificity, since *uidA* gene is also present in *Shigella* (Frampton and Restaino, 1993). The objective of the present study was therefore to develop a rapid detection method for *E. coli* in food samples, using a combination of enrichment and PCR that can also differentiate *E. coli* from *Shigella*.

Materials and Methods

Bacterial preparation and determination of detection limit

Five *E. coli* strains (*E. coli* NCCP11142, *E. coli* NCCP14037, *E. coli* NCCP14038, *E. coli* NCCP14039, and *E. coli* NCCP15661), and *Shigella sonnei* NCCP14743 strain were cultured in 10 mL tryptic soy broth (TSB, Becton, Dickinson and Company, USA). One-hundred microliter aliquots were transferred to fresh 10 mL TSB, followed by incubation at 37℃ for 24 h. The cultures of the five *E. coli* strains were mixed. Twenty-five milliliters of the *E. coli* mixture and 10 mL *S. sonnei* were centrifuged at 1,912 *g* and 4℃ for 15 min, and the pellets were washed twice with the same volume of phosphate-buffered saline (PBS; 0.2 g KH_2PO_4, 1.5 g Na_2HPO_4, 8.0 g NaCl, and 0.2 g KCl in 1 L distilled H_2O [pH 7.4]). The suspension was diluted with PBS to obtain 3, 4, and 5 Log CFU/mL of inocula, and *E. coli* and *S. sonnei* were assayed by PCR to determine the detection limit.

Food sample preparation and inoculation

Ham of pork and round of beef were purchased from a butcher shop, and a fresh-cut lettuce was purchased from a supermarket, located in Seoul, South Korea. Ham of pork and round of beef were cut into 20-g portions with a flame-sterilized knife. Fresh pork (20 g, n=4), beef (20 g, n=4), and fresh-cut lettuce (20 g, n=4) were placed aseptically into separate filter bags (3M, St. Paul, MN, USA). *E. coli* inoculum (0.1 mL) was inoculated onto the surface of the food samples to achieve 1, 2 and 3 Log CFU/g, and samples were massaged 20 times by hand. Samples were then left at room temperature (25℃) for 15 min to allow cell attachment.

E. coli enrichment in food samples

Eighty milliliters of EC broth (BD, USA) were placed into the filter bags, and shaken by hand 30 times. All samples were incubated at 44.5℃ (Feng et al., 2002) for 0, 4, and 5 h for pork and beef, or 0 and 3, 6, 12 h for fresh-cut lettuce. After enrichment, 1-mL aliquots of the enriched samples were plated onto *E. coli*/coliform petrifilm (3M, USA) to quantify *E. coli*. The plates were incubated at 37℃ for 24 h, and colonies were manually counted.

DNA extraction

One-milliliter aliquots of inocula and enriched samples were centrifuged at 18,341×g at 4℃ for 5 min, and supernatants

were discarded. Cell pellets were resuspended in 30 μL distilled water and boiled at 100℃ for 10 min, and the suspensions were centrifuged at 18,341×g and 4℃ for 3 min. The supernatants were then used for PCR analysis.

PCR analysis

Primers targeting the *uidA* and *Shigella* identification gene were used to differentiate *E. coli* from *Shigella* (Table 1). PCR conditions were as follows: 94℃ for 2 min (initial denaturation), 94℃ for 20 s (denaturation), 72℃ for 20 s (extension), and 72℃ for 2 min (final extension). Annealing was performed at 53℃ for *uidA* or at 62℃ for the *Shigella* identification gene for 10 s, and 35 cycles were performed. PCR analysis was performed using Fast mix French PCR (i-Taq) (iNtRon Biotechnology, Gyeonggi-do, Korea), and PCR products were run on an agarose gel (1.5%) with electrophoresis for 20 min. Target bands were visualized under UV light.

Results and Discussion

Minimum cell counts for PCR analysis, using primers for *uidA* gene were 3–4 Log CFU/mL for *E. coli* and 3 Log CFU/mL for *Shigella* (Fig. 1). From this result, we confirmed that 3–4 Log CFU/mL of bacterial cell counts was required to detect *E. coli* with primers targeting *uidA* gene, and the primers can detect both *E. coli* and *Shigella*. Hence, additional primers were necessary to differentiate *E. coli* from *Shigella*. Subsequently, the *Shigella* identification primers described in Table 1 were used, and the *Shigella* identification primers differentiated *E. coli* from *Shigella* (Fig. 1).

Analysis was then performed to determine optimum enrichment times required to obtain 3–4 Log CFU/mL of *E. coli* for PCR analysis. *E. coli* was inoculated into fresh pork, beef, or fresh-cut lettuce at 1, 2, and 3 Log CFU/g. *E. coli* in the pork and beef were enriched for 4 and 5 h, and *E. coli* in the fresh-cut lettuce were enriched for 3, 6 and 12 h. After 5-h enrichment, *E. coli* cell counts in the pork and beef increased to 5.9–6.0, 7.1, and 8.0–8.5 Log CFU/g for 1, 2, and 3-Log CFU/g inoculation levels, respectively, and *uidA* gene expression could be detected at all cell concentrations (Table 2, Fig. 2). In

Table 1. Primers for polymerase chain reaction (PCR) analysis

| Bacteria	Target gene	Size (bp)	Primer sequence (5′-3′)	Reference
Escherichia coli	*uidA*	252	PT-2, GCGAAAACTGTGGAATTGGG PT-3, TGATGCTCCATAACTTCCTG	Cebula et al. (1995)
Shigella	*Shigella* identification gene	159	F255, TCGCATTTCTCTCCCCACCACG F413, CCGGATGTGTCTCGGGCAATC	Kim et al. (2017)
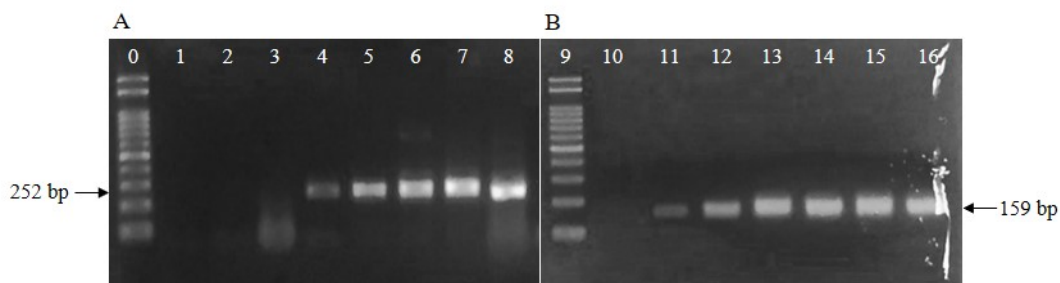
Fig. 1. **Detection of *Escherichia coli* (A, *uidA* without enrichment; lanes 1–8) and *Shigella* (B, *Shigella* identification gene without enrichment; lanes 10–16) by PCR.** Lanes 0 and 9: 100-bp ladder; lane 1: 1 Log CFU/mL cell counts; lanes 2 and 10: 2 Log CFU/mL cell counts; lanes 3 and 11: 3 Log CFU/mL cell counts; lanes 4 and 12: 4 Log CFU/mL cell counts; lanes 5 and 13: 5 Log CFU/mL cell counts; lanes 6 and 14: 6 Log CFU/mL cell counts; lanes 7 and 15: 7 Log CFU/mL cell counts; lanes 8 and 16: 8 Log CFU/mL cell counts.

Table 2. *Escherichia coli* cell counts (Log CFU/g, mean±SD) in fresh meats (pork and beef) and fresh-cut lettuce after 0, 4, and 5 h- and 0, 3, 6, and 12 h-enrichment with *E. coli* (EC) broth

| Food matrix	Targeted *E. coli* cell counts (Log CFU/g)	Enrichment time (h)					
		0	3	4	5	6	12
Pork	1	0.7±0.0	-[1]	4.7±0.0	5.9±0.5	-	-
	2	1.5±0.0	-	5.8±0.0	7.1±0.4	-	-
	3	3.1±0.0	-	6.9±0.0	8.5±0.1	-	-
Beef	1	0.8±0.2	-	4.4±0.6	6.0±0.6	-	-
	2	1.5±0.2	-	6.1±0.1	7.1±0.6	-	-
	3	3.0±0.0	-	7.3±0.1	8.0±0.2	-	-
Fresh-cut lettuce	1	1.0±0.2	4.2±0.2	-	-	6.3±0.0	7.2±0.0
	2	1.7±0.2	5.5±0.2	-		8.3±0.0	9.0±0.0
	3	3.1±0.2	6.5±0.1	-	-	8.4±0.0	8.0±0.0

[1] Not applied.
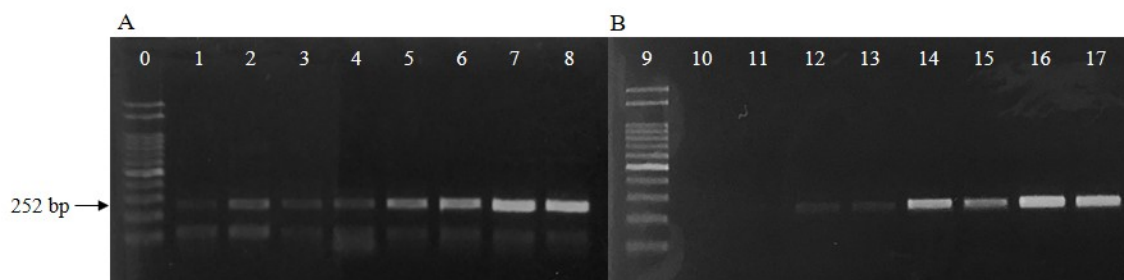
Fig. 2. Detection of *Escherichia coli* in fresh pork (A) and beef (B) samples by PCR for *uidA* after 5-h enrichment with *E. coli* (EC) broth. Lanes 0 and 9: 100-bp ladder; lanes 1, 2, 10, and 11: non-inoculated samples; lanes 3, 4, 12, and 13: 1-Log CFU/g inoculated samples; lanes 5, 6, 14, and 15: 2-Log CFU/g inoculated samples; lanes 7, 8, 16, and 17: 3-Log CFU/g inoculated samples.

fresh-cut lettuce after 3-h enrichment, the bacterial cell counts increased to 4.2, 5.5, and 6.5 Log CFU/g for 1, 2, and 3-Log CFU/g inoculation levels, respectively, and *uidA* gene was positive for all samples (Table 2, Fig. 3). Thus, the optimal enrichment time for PCR detection of *E. coli* was 5 h for fresh pork and beef, and 3 h for fresh-cut lettuce.

The above results show that meat samples require a longer enrichment time than fresh-cut lettuce. Low *E. coli* concentrations (0.7–0.8 Log CFU/g) in pork and beef increased to 4.4–4.7 Log CFU/g after 4-h enrichment, and the samples were
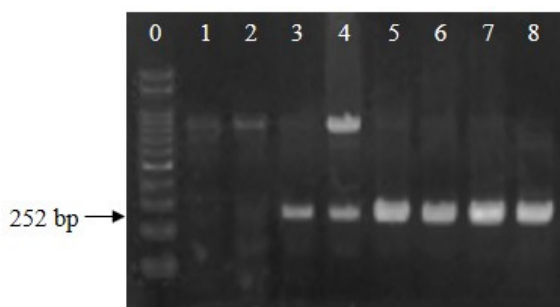
Fig. 3. Detection of *Escherichia coli* in fresh-cut lettuce samples by PCR for *uidA* after 3-h enrichment with *E. coli* (EC) broth. Lane 0: 100-bp ladder; lanes 1 and 2: non-inoculated samples; lanes 3 and 4: 1-Log CFU/g inoculated samples; lanes 5 and 6: 2-Log CFU/g inoculated samples; lanes 7 and 8: 3-Log CFU/g inoculated samples.

negative for *uidA* expression (Table 2). However, at similar *E. coli* concentrations in fresh-cut lettuce, the samples were *uidA* positive. It is possible that a component of the meat samples is interfering with the PCR analysis. Wang and Salazar (2016) showed that a number of intrinsic factors can interfere with PCR assays, and other studies have shown that particulates such as fats and carbohydrates can affect nucleic acid amplification (Dwivedi and Jaykus, 2011; Thomas et al., 1991). For this reason, extra pre-treatment, such as centrifugation and bead-based techniques, are necessary to remove some particles from certain foods (Rossen et al., 1992; Yang et al., 2007). Heidenreich et al. (2010) detected *E. coli* in ground beef using an electrochemical biochip method after enrichment for 4–5 h, and Li et al. (2017) used propidium monoazide treatment to detect viable cell counts of *E. coli* O157:H7 at 12-h enrichment. However, in this present study, 5-h enrichment for fresh meat samples and 3-h enrichment for fresh-cut lettuce were sufficient to detect *E. coli* by PCR.

In conclusion, the combination of enrichment and PCR detection method is able to detect *E. coli* via applying PCR with *uidA* primers to samples directly after 5-h enrichment for fresh meats (pork and beef) and 3-h enrichment for fresh-cut lettuce.

Acknowledgements

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