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SHORT COMMUNICATION

# 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°C, 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°C, 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

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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<br>PT-3, TGATGCTCCATAACTTCCTG | Cebula et al. (1995) |
| *Shigella* | *Shigella* identification gene | 159 | F255, TCGCATTTCTCTCCCCACCACG<br>F413, CCGGATGTGTCTCGGGCAATC | Kim et al. (2017) |

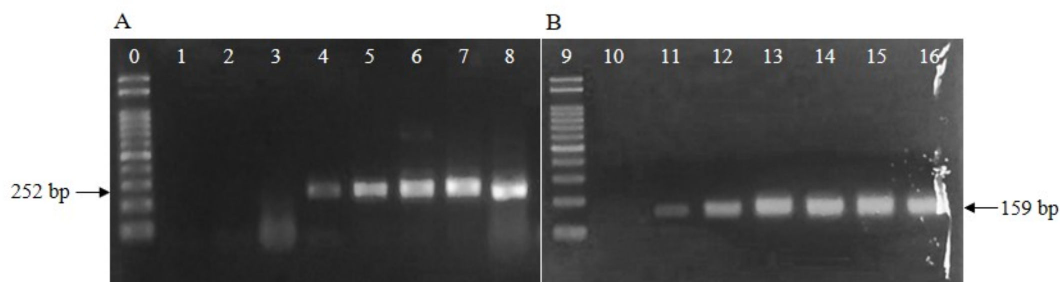


**Fig. 1. Detection of *Escherichia coli* (A, *uidA* without enrichment; lanes 1–8) and *Shigella* (B, *Shigella* identification gene without enrichment; lanes 10–16) by PCR.** Lanes 0 and 9: 100-bp ladder; lane 1: 1 Log CFU/mL cell counts; lanes 2 and 10: 2 Log CFU/mL cell counts; lanes 3 and 11: 3 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.

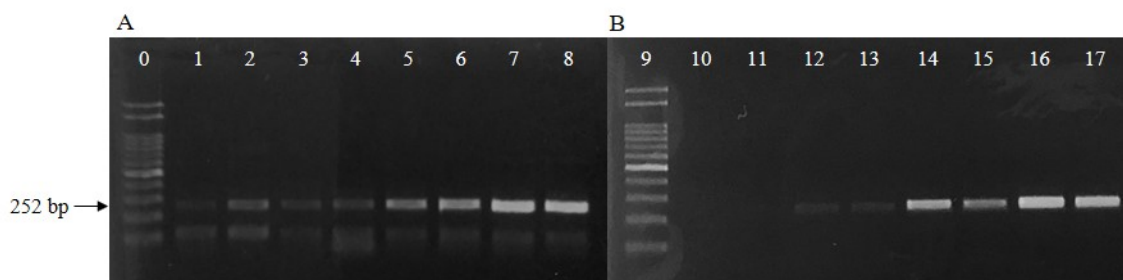


**Fig. 2. Detection of *Escherichia coli* in fresh pork (A) and beef (B) samples by PCR for *uidA* after 5-h enrichment with *E. coli* (EC) broth.** Lanes 0 and 9: 100-bp ladder; lanes 1, 2, 10, and 11: non-inoculated samples; lanes 3, 4, 12, and 13: 1-Log CFU/g inoculated samples; lanes 5, 6, 14, and 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

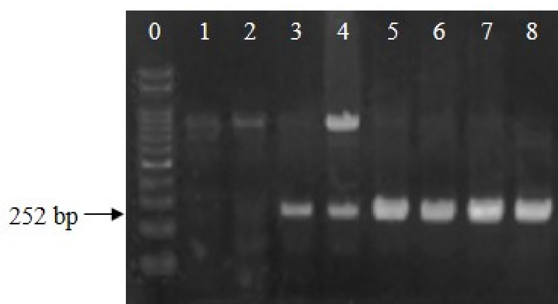


**Fig. 3. Detection of *Escherichia coli* in fresh-cut lettuce samples by PCR for *uidA* after 3-h enrichment with *E. coli* (EC) broth.** Lane 0: 100-bp ladder; lanes 1 and 2: non-inoculated samples; lanes 3 and 4: 1-Log CFU/g inoculated samples; lanes 5 and 6: 2-Log CFU/g inoculated samples; lanes 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.

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