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PCR Method Based on the *ogdH* Gene for the Detection of *Salmonella* spp. from Chicken Meat Samples

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In a previous paper, the *ogdH* gene that encodes 2-oxoglutarate dehydrogenase was isolated from *Salmonella typhimurium*. The catalytic *N*-terminal region in the enzyme was found to be very specific for the Salmonella species. Therefore, the aim of the present study was to detect *S. typhimurium* in food sources using primers designed for OGDH-1 and OGDH-2 which were based on the salmonella-specific region of the *ogdH* gene. A simple polymerase chain reaction (PCR) detection method was developed to detect low numbers of *S. typhimurium* in a chicken meat microbial consortium. Using the *ogdH*-specific primers under stringent amplification conditions and for gene probe analysis, fewer than 100 colony-forming units (CFUs) were detectable when pure cultures were employed. When the PCR assay was run on *S. typhimurium*-contaminated meat contents, only the positive meat samples containing as few as 200 CFUs reacted to the assay. The method employed for sample processing is simple and it was determined to provide a sensitive means of detecting trace amounts of *S. typhimurium*-specific sequences in the presence of mixed meat microbial populations. When compared with six representative intestinal gram-negative bacterial strains in foods, including *Vibrio parahaemolyticus, V. vulnificus, Enterobacter cloacae, E. coli* O157:H7, *Pseudomonas aeruginosa,* and *Proteus* sp., *S. typhimurium* had a unique and distinct PCR product (796 bp). In conclusion, the two OGDH primers were found to be rapid and sensitive detectors of *Salmonella* spp for the PCR method.

Key words: PCR, 2-oxoglutarate dehydrogenase, functional domain, *(Salmonella typhimurium)*

Salmonella typhimurium has been recognized as an important worldwide infectious disease in humans and animals for over 50 years. Concurrently, the recognition of Salmonellosis (typhoid fever) as an important public health problem has also been recognized, but this only dates back from the documentation of common-source food borne outbreaks in the past few decades. It has been reported that *Salmonella* species occur in meat, poultry, meat products, and raw milk etc. (Greenwood and Hooper, 1983; D'Aoust, 1985). Typhoid fever caused by the bacterium may frequently occur by eating raw vegetables; however, the bacteria are usually destroyed by cooking.

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Recently, tests based on DNA restriction enzyme analysis, southern hybridization, and a polymerase chain reaction (PCR) method have been used to detect *S. typhimurium* in food and blood samples (Ezpuerra *et al.*; Fitts, R. 1985; Knight *et al.*, 1990). In each of these processes, the inoculation of a nutrient broth with food samples is a common step. Classical techniques require multiple subcultures with various selective and indicator media. These methods may take as long as a week to complete and are limited by poor sensitivity (Bej *et al.*, 1994).

The standard culture method recommended by the Association of Official Analytical Chemists for the isolation of *Salmonella* from foods consists of a 24 h pre-enrichment, a 24 h selection enrichment, and 24-to-48 h selective plating followed by biochemical/serological confirmation of suspect colonies (Pillai *et al*, 1994). In addition to the delays associated with the culture methodologies, tradi-

tional plating techniques can significantly underestimate the actual numbers of pathogens in any environment (Pillai *et al*, 1994). In various foods, it is still difficult to detect *S. typhimurium* even though many successful approaches for rapid detection have been reported (Fitts, 1985); nonetheless, other contaminated microbes can influence the sensitivity of detection. Thus, any detection method for *Salmonella* spp. requires the ability not only to rapidly screen samples and their environment, but to do so with samples containing highly diverse and transitional populations of bacteria. The use of gene amplification methods such as PCR to detect minimal pathogens in food circumvents the need to use cultures for screening purposes.

This study reports a simple assay that includes primers (OGDH-1 and -2) targeted against 2-oxoglutarate dehydrogenase (an enzyme of the TCA cycle dehydrogenase system), which regulates the expression of genes involved in the energy production of *S. typhimurium* (Kim, 2003). The 2-oxoglutarate:lipoamide dehydrogenase (OGDH, decarboxylating and acceptor-succinating or E1o. EC 1.2.4.2) multienzyme complex (ODHC) catalyzes the oxidative decarboxylation of 2-oxoglutarate to succinyl coenzyme A, a reaction which is part of the citric acid cycle (Pettit *et al.*, 1973; Darlison *et al.*, 1984; Ricaud *et al.*, 1996). In the previous study by Kim (2003), the catalytic domain of the clone enzyme was localized in the *N*-terminal region of the gene and the region was found to be very specific for the *Salmonella* species. Therefore, we designed two PCR primers that can be used to detect *S. typhimurium* from several important and six representative intestinal microbes in food. The objective of this study was to develop a rapid sample processing method to aid in the screening of *S. typhimurium* in the presence of a diverse meat microbial consortium using OGDH-1 and -2 primers, which were based on the salmonella-specific region of the *ogdh* gene.

Materials and Methods

Materials

Restriction and modification enzymes were purchased from BMS (Korea), and were used as recommended by the suppliers. Molecular marker kits for agarose gel electrophoresis were obtained from Bio-Rad Laboratories (Seoul, Korea). A radioisotope for DNA hybridization was purchased from Amersham Korea (Korea). Other chemicals used, including EDTA, were of the purest grade commercially available.

Bacterial strains, media and culture

The reference strains of salmonellae including *S. typhimurium* (ATCC 14028) and wild types isolated from poultry such as *S. enteritidis, S. montevideo,* and *S. muenchen* are shown in Table 1, as previously described (Jin *et al.*, 1999; Jin *et al.*, 2001; Won *et al.*, 2001; Shin *et al.*, 2002). The six representative intestinal gram-negative bacterial strains in foods, including *V. parahaemolyticus* (ATCC 27519), *V. vulnificus* (ATCC 29307), *Enterobacter cloacae* (ATCC 13047), *E. coli* O157:H7, *P. aeruginosa* (ATCC 27582), and *Proteus* spp. (N 13838) were obtained from Dr. SD Ha Korean Health Industry Development Institute (KHIDI), Korean Ministry of Health and Welfare (KMHW). *S. typhimurium* LT2 (SGSC SL3770) was supplied by Dr. Ken Sanderson from the Salmonella Stock Center (SSC), University of Calgary, Calgary, Alberta, Canada. *S. typhimurium* was grown on tryptic soy broth (TSB, Difco, USA) containing 20 μg/ml of nalidixic acid (NA) and novobiocin (NO) (Sigma, Korea), other strains were grown on Luria broth (1% peptone, 0.5% yeast extract and 0.5% NaCl, pH 7.2).

A primary poultry and meat isolate of *S. typhimurium* obtained from the Korea Food Research Institute (Kyunggi, Korea) was selected for its resistance to NO and NA in our laboratory and maintained in media containing 20 μg/ml of nalidixic acid and novobiocin (Sigma, USA). Other strains were grown on LB broth. *S. typhimurium* (wild type) strains were grown in TSB or on brilliant green agar (BGA, Difco, USA) containing 25 μg/ml of NO and NA at 37°C. Challenge inocula were prepared from an overnight culture that had been previously maintained with tryptic soy broth (Difco, USA) and then serially diluted in sterile phosphate-buffered saline (PBS) to a concentration of 3×10^4 colony-forming units (CFU) per ml (Jin *et al.*, 2001). The viable cell concentration of each challenge inoculum was confirmed by colony counts on brilliant green agar plates containing 20 μg/ml of NO and 20 μg/ml of NA.

Meat sample preparation for Salmonella detection and bacterial DNA isolation from meat sources

For the PCR assay of *S. typhimurium* inoculated in food, chicken purchased from a local market was minced and 5 g of a sample containing skin and other meat parts was suspended in 20 ml of TSB. *S. typhimurium* was grown in TSB for 36 h then inoculated into TSB containing minced chicken to a final concentration ranging from 1.3 to 1.3×10^5 CFU/ml. These chicken-TSB mixtures were incubated for 24 h at 37°C with gentle shaking for bacterial enrichment. Meats were inoculated with 1×10^4 CFU *S. typhimurium* NO/NA. Meat samples were randomly selected from the inoculated meats after 12 h, stored at 4, and then screened for *S. typhimurium* (Jin *et al.*, 1999).

In contrast, for time-coursed sampling, samples were obtained 0, 4, 8, 12 and 24 h after a meat-TSB incubation at 37°C with gentle shaking. One ml of each sample was filtered using sterile filter paper (Whatman No. 1) and centrifuged at 10,000 rpm for 5 min. The resulting pellet was washed twice with PBS and resuspended in 0.1 ml of PBS. One μl of the cell suspension was used for PCR amplification, indicating that the cell number used for the PCR reaction was 1/100 of the original culture broth per ml. The samples were lysed with 0.1 mg/100 μl (final concentration) proteinase K (Merck, Germany) for 60 min

at 60°C and finally boiled for 15 min. A 10 μl volume of this lysate was subjected to PCR analysis without further purification (Won *et al.*, 2001).

Bacterial DNA isolation from pure culture

DNA samples were purified from the bacterial strains by using the standard protocol of Maniatis *et al.* (1982) and Takara Biotechnology (Japan); DNA samples were treated with lysozyme and STET solution (0.5% SDS, 50 mM Tris-HCl, pH 8.0, 0.4 M EDTA, 1 mg/ml proteinase K), repeatedly extracted with phenol-chloroform-isoamylalcohol (25 : 24 : 1, v : v : v), followed by a final extraction with chloroform-isoamylalcohol (24 : 1, v : v). The DNA was concentrated by precipitation with absolute ethanol and treated with RNase A.

Oligonucleotide primers

The previous study by Kim (2003) showed that the OGDH-1 and -2 primers can specifically amplify the 796-bp region of the *ogdh* gene, and specifically detect *S. typhimurium* when used in an amplification protocol. Our preliminary studies demonstrated that under stringent application conditions, OGDH primers could selectively amplify *Salmonella* spp.-specific nucleic acids. Thus, the 21-mer OGDH primer pair (OGDH-1 for forward direction: 5'-GCCTTCCTGAAACGTGACCTA-3'; -64 to -45) and OGDH-2 for reverse direction: 5'-ACCATCTC-TTTCAGCATGGGT3'; +708 to +728) was synthesized using a DNA sunthesizer (Model No. 380A, Applied Biosystems, USA) and employed in PCR amplification.

PCR assays

Pure culture. A 24 h broth culture of the *S. typhimurium* NO/NA strain was prepared in Luria broth. Serial dilutions of the culture were made, and aliquots (100 μl) of the dilutions were used for PCR amplifications and for enumerating the total number of viable cells using plating methods. For the PCR amplifications, 100 μl of the dilutions were centrifuged at 15,000 rpm for 10 min, the supernatant was discarded, and the cell pellet was used in the reaction. The DNA present within the cells in the pellet served as the template.

Primer specificity. In order to confirm the specificity of the OGDH primers against *S. typhimurium,* NO/NA and genomic DNAs were used as a template for the amplification. Briefly, 10 ml of the broth culture was pelleted, and the pellet was resuspended in 500 μl of Tris-EDTA-glucose buffer (25 mM Tris, 10 mM EDTA, 50 mM glucose, pH 8.0). To this, 500 μl of lysozyme (8 mg/ml) was added, and the suspension was incubated on ice for 10 min. One hundred microliters of 10% SDS was added, and the suspension was incubated for another 10 min. Proteinase K (1 mg/ml) was freshly prepared, and 60 μl was added to the above suspension. This suspension was shake-incubated for 3 h at 50°C, after which the DNA was phenol-chloroform isoamylacohol extracted and ethanol precipitated at -20°C.

PCR amplification and conditions. Gene amplification of the target sequence was carried out using the Takara's GeneAmp kit with *Taq* DNA polymerase in a DNA thermal cycler (Takara Shuzo, Japan) since Ampli*Tag* DNA polymerase does not extend primers across template DNA if there is even a single nucleotide mismatch at the 3'-OH ends (Shin *et al.*, 2002). The reaction mixture contained 0.3 μg of each chromosomal DNA, 10 mM Tris-HCl (pH 8.3), 5 mM KCl, 1.5 mM $MgCl_2$, 100 mM of each dNTP, 25 pmol of OGDH-1 and -2 primers, and 0.5 U of *Taq* DNA polymerase (Takara, Japan). The mixture was over-

Sense primer (OGDH-1; -65 to -44)

Agcttgcc**gccttcctgaaacgtgacctat**ttagagtattaaataagcagaaaagatgcttaagggatcacg**ATG**cagaacagcgctttgaa

agcctggttggactcttcttacctctctggttcgaatcagagctggatagaacagctctatgaagacttcttaaccgatcctgactcggtagacgct

aactggcgtttgacgttccagcagttacctggtaccggagtcaaaccggatcaactccattcaaaaacacgtgaatatttccggcggcaggcgtt

ggctggctcacgtcactcttctacgatttccgaccctgacaccaatgtgaagcaggttaaagtcctgcagcttatcaacgcttatcgtttccgtggc

catcaacatgcaaacctcgatccgctgggactgtggaagcaagaacgcgtggcggatctcgatccttctttccatgatttgaccgaggccgattt

ccaggaaaccttgaatgtcggctcctttgccagcggcaaagagacgatgaagctgggcgagctgctcgacgcgctcaaacagacctactgcg

gcccgattggcgctgagtatatgcacatcaccagcaccgaagagaaacgctggatccaacagcgcatagaatccggtcgtgcggcctttagcg

ctgacgagaaaaaacgcttcctgaacgaactgaccgccgctgaagggctggaacgttatctgggcgccaaattcccgggtgcgaaacgtttct

cgctcgaggggggagatgcgctgat**acccatgctgaaagagatggt**tcgccatgcgggtaacagcggcactcgcgaagtggtgctgggg

Antisense primer (OGDH-2; +708 to +728)

atggcgcaccgcggacgcctgaacgtgctgatc

Fig. 1. Nucleotide sequence of the PCR product produced from the *S. typhimurium ogdH* gene. (A) The 0.9-kb sequence contains the 5'-starting region of the *ogdH* gene. The nucleotide at position 1 corresponds to the first nucleotide in the *Hin*dIII recognition sequence. The nucleotide sequence of the *ogdH* gene has been deposited in the GenBank database (accession no. AF093783).

laid with 50 μl of mineral oil (Sigma, USA), and the PCR was carried out in a Takara PCR thermal cycler 480. The temperature cycling was as follows: after an initial denaturation at 95°C for 5 min, the subsequent 45 cycles consisted of a denaturation at 95°C for 1 min, an annealing of the primer at 36°C for 1 min, and an extension at 72°C for 2 min without a final extension step during the last cycle. The PCR products were separated by gel electrophoresis in 1.5% agarose (Sigma, USA) gels containing ethidium bromide with 1×TAE buffer, and visualized under a UV illuminator.

Results and Discussion

Design of the PCR amplification primers specific for S. typhimurium detection

Previously, a 2.8-kb DNA fragment carrying the *ogdH* gene was cloned and the structure gene for the protein was sequenced (Kim, 2003). Our preliminary studies demonstrated that under stringent application conditions, OGDH-1 and -2 primers could selectively amplify *S. typhimurium*-specific nucleic acids. It was shown that the OGDH primers can specifically amplify the 796-bp region of the *ogdH* gene, and specifically detect *S. typhimurium* when used in an amplification protocol. In the present study, a 796 bp fragment within the region of *ogdH* that is specific for the *S. typhimurium* DNA was targeted for PCR amplification. Thus, the 21-mer OGDH primer pair (OGDH-1; 5'-GCCTTCCTGAAACGTGACCTA-3' and OGDH-2; 5'-AGTAGAGAAACTCGTAGCCC-3') was synthesized and employed in the PCR amplification as shown in Fig. 1.

Specificity of the designed PCR amplification using Salmonella species and six representive intestinal gram-negative bacterial strains

S. typhimurium is frequently isolated together with other intestinal gram-negative bacterial strains in foods, such as *V. parahaemolyticus, V. vulnificus, E. cloacae, E. coli* O157:H7, *P. aeruginosa,* and *Proteus* sp.., The specificity

Table 1. Results of PCR based on the OGDH sequence and reference strains tested

Species	Result of PCR with primers (+ or −)	Size of DNA band (bp)	Sources
S. typhimurium (ATCC 14028)	+[a]	796	KMHW[b]
S. typhimurium A (Wild type from poultry)	+	796	KMHW
S. typhimurium B (Wild type from poultry)	+	796	KMHW
S. typhimurium C (Wild type from poultry)	+	796	KMHW
S. typhimurium D (Wild type from poultry)	+	796	KMHW
S. typhimurium E (Wild type from poultry)	+	796	KMHW
S. typhimurium F (Wild type from poultry)	+	796	KMHW
S. typhimurium G (Wild type from poultry)	+	796	KMHW
S. typhimurium H (Wild type from poultry)	+	796	KMHW
S. typhimurium I (Wild type from poultry)	+	796	KMHW
S. enteritidis (Wild type from poultry)	+	796	KMHW
S. montevideo (Wild type from poultry)	+	796	KMHW
S. muenchen (Wild type from poultry)	+	796	KMHW
S. paratyphi 8A39	+	796	KMHW
S. typhimurium DPD21	+	796	KMHW
S. typhi (ATCC 6539)	+	796	KMHW
S. typhimurium LT2 (SGSC SL3770)	+	796	SGSC[c]
V. parahaemolyticus (ATCC 27519)	−		KMHW
V. vulnificus (ATCC 29307)	−		KMHW
Enterobacter cloacae (ATCC 13047)	−		KMHW
E. coli O157:H7	−		KMHW
P. aeruginosa (ATCC 27582)	−		KMHW
Proteus spp. (N 13838)	−		KMHW

[a]− and + denote the amplified PCR band (793 bp) as negative or positive, respectively.
[b]KMHW, Korean Ministry of Health and Welfare.
[c]SGSC, Salmonella Genetic Stock Center, University of Calgary, Alberta, Canada.

of the OGDH-1 and OGDH-2 primers was tested for PCR amplification of the DNA isolated from the strains listed in Table 1. The PCR amplification generated the characteristic 796-bp band patterns for all of the *S. typhimurium* samples tested. For the PCR amplification applied to other strains, the characteristic 796-bp band was not observed (Fig. 2A). The results summarized in Table 1 indicate that a PCR amplification using oligonucleotide primers of OGDH-1 and OGDH-2 provides a very specific means of identifying all *S. typhimurium* strains including clinical isolates. Since current techniques using selective media fail to provide optimal differentiation of *S. typhimurium* from other intestinal gram-negative bacterial strains in foods, it is especially important to develop a detection method that provides such specificity. From this result, it is suggested that this PCR method that uses primers of OGDH-1 and OGDH-2 can be a potentially useful tool for the rapid and direct identification of *S. typhimurium* naturally present in food samples.

This study also analyzed 12 strains of salmonellae for specificity. The PCR with the two primers amplified the DNA from all *S. typhimurium* strains, *S. enteritidis* *S. Enteritidis*, *S. montevideo,* and *S. muenchen* (Table 1). A band migrating close to the predicted 796-bp size was detected by agarose gel electrophoresis and ethidium bromide staining (Fig. 2B).

Detection condition of S. typhimurium in inoculated chicken meat

PCR was performed for the detection of. *S. typhimurium* in inoculated chicken meat sources using oligonucleotide primers of the OGDH-1 and OGDH-2 pair, as shown in Table 2. The initial levels of 1.3 to 1.3×10^4 CFU/ml of *S. typhimurium* rose to 10^2 to 10^6 CFU/ml after 12 h and to

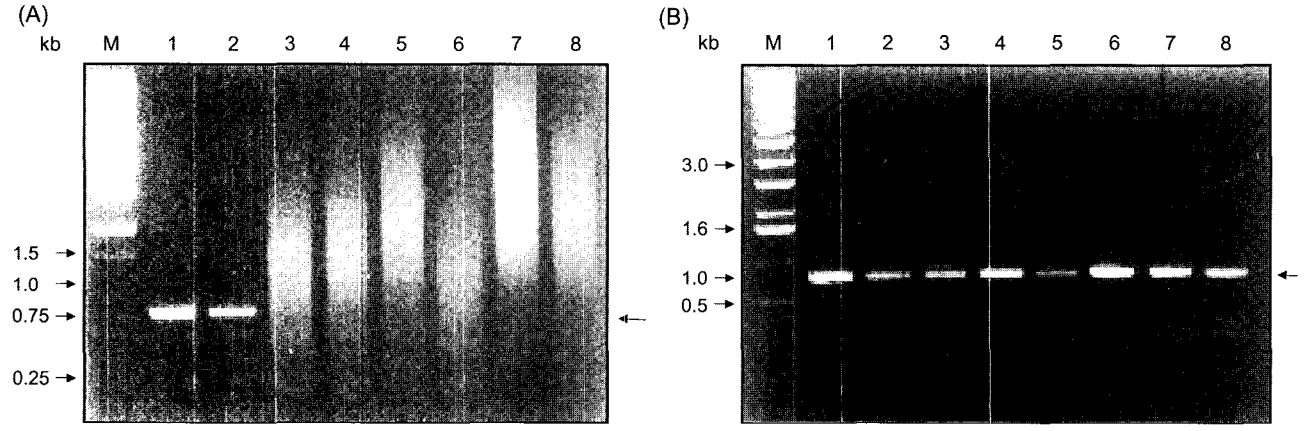

Fig. 2. (A) Specificity of primers and agarose gel electrophoresis of the PCR product using the primers designed from the *S. typhimurium ogdH* gene with six representative intestinal gram-negative bacterial strains. Lane M, DNA size marker (Boehringer mannheim marker X); 1, plasmid pSH2-08 DNA carrying the *ogdH* gene; 2, *S. typhimurium* DNA (wild type, poultry isolate); 3, *V. parahaemolyticus* ATCC 27519; 4, *V. vulnificus* ATCC 29307; 5, *Enterobacter cloacae* ATCC 13047; 6, *E. coli* O157 : H7; 7, *Pseudomonas aeruginosa* ATCC 27582; 8, *Proteus* sp. N 13838.
(B) Agarose gel electrophoresis of the PCR product using the primers designed from the *S. typhimurium ogdH* gene with *Salmonella* species. Lane M, DNA size marker (StratageneR); 1, *S. typhimurium* (ATCC 14028); 2, *S. typhimurium* A (wild type from poultry); 3, *S. typhimurium* B (wild type from poultry); 4, *S. typhimurium* C (wild type from poultry); 5, *S. typhimurium* D (wild type from poultry); 6, *S. Enteritidis* (wild type from poultry); 7, *S. montevideo* (wild type from poultry); 8, *S. muenchen* (wild type from poultry).

Table 2. PCR detection of *S. typhimurium* in inoculated chicken meat with time course

Initial inoculum (CFU/ml)	PCR detection (796 bp)					Cell number (CFU/ml)				
	Incubation time (h)					Incubation time (h)				
	0	4	8	12	24	0	4	8	12	24
0	–[a]	–	–	–	–	0	0	0	0	0
1.3×10^0	–	–	–	–	–	0	0	10	10^2	10^3
1.3×10^1	–	–	–	+	+	10^1	10^1	10^2	10^3	10^4
1.3×10^2	–	+	+	+	+	10^2	10^2	10^2	10^4	10^5
1.3×10^3	–	+	+	+	+	10^3	10^3	10^4	10^5	10^7
1.3×10^4	+	+	+	+	+	10^4	10^4	10^5	10^6	10^8

[a] – and + denote the amplified PCR band (793 bp) as negative or positive, respectively.

10^3 to 10^8 CFU/ml after 24 h, respectively. The 796-bp PCR products were generated from all samples. The detection limit was at the level of 10 - 10^2cells for the pure culture and chicken samples, indicating that the detection limit when using the OGDH-1 and OGDH-2 primers is not affected by the food composition or enriched food samples. Although the chicken enrichment samples were a mixture of crude chicken meat and released fragment components solved in TSB, the PCR detection method for *S. typhimurium* was shown to be sensitive. The problem lies in that the number of foodborne pathogens usually present in food sources is at much lower levels than that present in a general PCR condition (Shin *et al.*, 1999; Olsen *et al.*, 1995). Therefore, it is generally accepted that direct PCR detection is technically impossible at the 1-10 cell numbers per PCR level (Shin *et al.*, 1999).

Sensitivity for detection of S. typhimurium in chicken meat

In order to determine the detection sensitivity of the PCR, each DNA isolated from the meat homogenate, seeded with *S. typhimurium* at an initial level of 10^3 CFU/ml, and incubated for 12 h, was used for the extraction of DNA. Then, each reversed DNA was used as a template for PCR. The sensitivity for detecting *S. typhimurium* present in meat was tested by seeding homogenized meats with 10 to 10^4 CFU of *S. typhimurium* per ml of homogenate. After an incubation for up to 12 h, DNA was isolated from 1 ml of each homogenate and a 2 μl portion of the total DNA dissolved in 20 μl distilled water was used as a template for PCR amplification. In the homogenates seeded with 10^3 or more *S. typhimurium* CFU/ml, positive signals were obtained after an incubation of 12 h or less. From the samples inoculated with approximately 10^2 CFU/ml of the homogenate then incubated as long as 12 h, the targeted sequence was successfully amplified by PCR and the 796-bp DNA fragment was observed. 10^3 CFU cells corresponded to as few as 10 pg of DNA (lane 5). Interestingly, the samples seeded at initial levels of 10^1 CFU/ml to 1×10^2 CFU/ml of the homogenate (lane 2 and 3), showed the faint 796 bp band when incubated for 12 h (Fig. 3).

Although the exact concentration of *S. typhimurium* after incubation was not known, based on the detection of 10^2 CFU/ml at an initial level, the sensitivity of the procedure seems quite high. Recently, a sensitivity of 10^2 CFU/ml of *S. typhimurium* at an initial level has been reported when a *phoA* gene sequence is used as a primer (Pillai *et al.*, 1994). It has also been proposed that a higher sensitivity of detection for *S. typhimurium* can be obtained by optimizing the PCR method.

In this study, we developed a simple method that was found to definitively identify the *Salmonella* species by using short DNA primers based on the *ogdH* gene. PCR using the primers produced the characteristic and specific band for *S. typhimurium* strains. In the genomic DNAs of

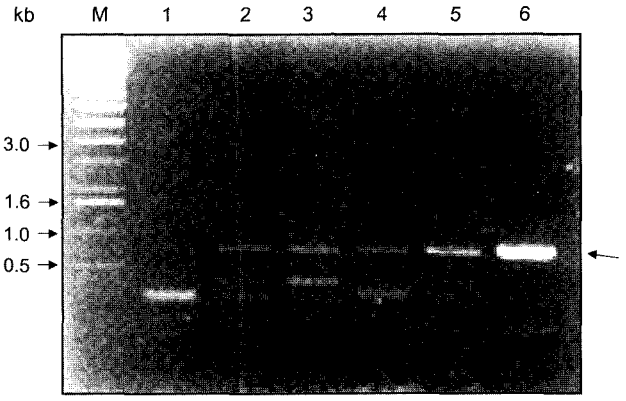

Fig. 3. PCR detection of *S. typhimurium* in chicken meats inoculated with the strain. Meat homogenates seeded with *S. typhimurium* at an initial level of 102 CFU/ml and incubated for 12 h were used for the extraction of DNA, and then each reversed DNA was used as a template for PCR. The migrations of standard molecular weights are indicated to the left in kb as molecular weight (lane M). Lanes 1, sample # 1 (0 CFU); 2, sample # 1 (1×10 CFU); 3, sample # 3 (1×10^2 CFU); 4, sample # 4 (2×10^2 CFU); 5, sample # 5 (1×10^3 CFU); 6, sample # 6 (1×10^4 CFU).

V. parphaemolyticus, V. vulnificus, Enterobacter cloacae, E. coli O157 : H7, *P. aeruginosa* and *Proteus* sp., there were no amplified DNA bands. The *S. typhimurium* DNA band pattern (796 bp) was different and characteristic in the PCR amplification, and the control bacterial strains did not produce any amplification with the primers used in this study, which confirmed the specificity of these primers. Even though there are several methods available that specifically detect *S. typhimurium* (Pettit *et al.*, 1973; Darlison *et al.*, 1984; Fitts, 1985; Knight *et al.*, 1990; Ezpuerra *et al.*, 1993; Bej *et al.*, 1994), an urgent need in the practice of *Salmonella* diagnosis is to develop a rapid, sensitive, reproducible, and reliable method.

It has been suggested that the major obstacle in using PCR on food samples is the presence of components that inhibit the polymerase activity or binding of primers (Dodd *et al.*, 1990; Koch *et al.*, 1993). A good separation procedure to remove the harmful substances from contaminated DNA is necessary to increase the sensitivity in PCR. Even though the nature of impurities is not yet known, our results strongly suggest that the present DNA extraction method is applicable for PCR. Since isolating a DNA template from samples is the most time consuming factor in PCR, developing an efficient DNA extraction method will reduce the time required for completion of the total procedure of identifying microorganisms in foods. Thus, the PCR method in this study, which addresses an urgent need in the practice of *Salmonella* diagnosis, fulfills all of the conditions for the characterization of *S. typhimurium.* Furthermore, it was clearly shown to differentiate *Salmonella* spp. from other intestinal bacterial strains present as contaminants in foods.

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