J. Fd Hyg. Safety
Vol. 22, No. 4, pp. 248~256 (2007)

Journal of Food Hygiene and Safety
Available online at http://www.foodhygiene.or.kr

Rapid Enumeration of *Salmonella* spp. in Contaminated Pork Meat Using Competitive PCR

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Competitive PCR을 이용한 돼지고기 오염 살모넬라의 신속 계수

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(Received September 23, 2007/Accepted December 5, 2007)

ABSTRACT – In this study, the competitive polymerase chain reaction (cPCR) was used to develop a direct enumeration method of *Salmonella* spp. in pork meat. After comparing three DNA extraction methods, the modified guanidine thiocyanate-phenol-chloroform method was chosen for *Salmonella* DNA extraction in artificially inoculated pork meat. The previously reported 284-bp *invA* gene (Rahn et al. Mol. Cell. Probes 1992) was tested for specificity, and 57 *Salmonella* strains and 24 non-*Salmonella* strains were evaluated. All *Salmonella* strains tested were *invA* positive, and all non-*Salmonella* strains produced no false positive amplification products. The detection limit achieved was as low as 1,460 colony-forming units (cfu) per 0.1 g of pork meat. For cPCR, the *invA* gene, which features a 82 bp-deletion, was cloned in the pGEM-4Z vector. A known amount of competitor DNA, which has the same primer binding sites, was co-amplified with *Salmonella* chromosomal DNA from the artificially inoculated pork meat. The cell-number determined by cPCR was approximately equal to the cfu from the most probable number (MPN) method. Finally, the whole procedure took only 5 hr.

Key words: Detection, Enumeration, *Salmonella* spp., PCR

Introduction

Salmonella spp. are members of the *Enterobacteriaceae*, and are divided into seven subspecies based on their biochemical characteristics. Serotyping has identified more than 2,000 serovars[1]. *Salmonella* spp. have been isolated from a variety of foods. The ingestion of contaminated food can cause salmolellosis. Thus, a rapid detection and enumeration method will play an important role for epidemiological surveillance, monitoring of food-borne outbreaks, and the identification of sources of contamination in food processing plants.

Detection and enumeration of *Salmonella* continues to be an important issue in clinical and applied microbiology, and new media and diagnostic tests are continuously being developed[2,3]. Biochemical substrate utilization is the basis of species identification for *Salmonella*. The majority of *Salmonella* spp. are recognized as non-lactose fermenters (Lac^-) and hydrogen-sulfide producers (H_2S^+). However, because the majority of the H_2S^+ and/or Lac^- colonies turn out not to be *Salmonella* spp., but insteads are related species such as *Proteus* or *Citrobacter*, conventional approaches require confirmatory testing of all H_2S^+ and/or Lac^- colonies, which extends the time for identification[3]. Traditional culture methods for the detection of *Salmonella* spp. require about 5 days to obtain a negative result, and it takes at least 2 days for clinical laboratories to give a positive report[4-6].

Several polymerase chain reaction (PCR) assays, not dependent on substrate utilization or the expression of antigens and thereby circumventing the phenotypic variations, have been developed to detect *Salmonella* spp. Regions that have been selected for PCR detection include *agfA*, *fimA*, *hin*, *H-li*, *iagAB*, *IS200*, *invA*, *iroB*, *mkfA*, *ompC*, *oriC*, *spvR*, *viaB*, *fimY*, *rfb*, *pefA*, *sefA*, *iagA*, *mntH*, *gyrA*, and *prot6e*[3,7-25]. However, limited data are available on the performance of PCR assays for the detection of *Salmonella*

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in foods.

Recently, we reported that competitive PCR (cPCR) can be applied to directly estimate the numbers of *Listeria monocytogenes*[26], *Salmonella enteritidis*[27], and *Yersinia enterocolitica*[28] in milk, and *Listeria monocytogenes* in pork meat[29]. In this study we evaluated the specificity of primers based on the invasion (*invA*) gene that is located on pathogenecity island 1 of *Salmonella* spp., which encodes a protein of the type III secretion system[30] for *Salmonella* detection, and then utilized it for the enumeration of *Salmonella* in pork meat using cPCR.

Materials and Methods

Bacterial strains

The *Salmonella* strains (Table 1) were obtained from the Seoul Metropolitan City Institute of Health and Environment (Seoul, Korea) and Korean National Institute of Health (Seoul, Korea) and were grown in Luria-Bertani (LB) medium containing 1% tryptone (Becton Dickinson, Sparks, MD, USA), 0.5% yeast extract (Becton Dickinson), and 1% sodium chloride (Sigma, St. Louis, MO, USA). The non-*Salmonella* strains used in this study are listed in Table 2. *Listeria* strains were obtained from the National Veterinary Research and Quarantine Service (Anyang, Korea) and were grown in TSBY containing Tryptic soy broth and 0.6% yeast extract (Becton Dickinson, Sparks, MD, USA). The *Escherichia coli* (*E. coli*) strains, except *E. coli* O157 H:7 ATCC43888 and *E. coli* K12 KCTC1041, were from the National Veterinary Research and Quarantine Service (Anyang, Korea). *E. coli* O157 H:7 ATCC 43888 and *Yersinia enterocolitica* ATCC 27729 were obtained from the Korean National Institute of Health (Seoul, Korea). *E. coli* K12 KCTC 1041 (ATCC 23736), *Shigella flexneri* ATCC 9199 (KCTC 2008), and *Shigella sonnei* ATCC 9290 (KCTC 2009) were obtained from the Korea Research Institute of Bioscience and Biotechnology (Daejeon, Korea).

Reagents

Restriction enzymes and a Wizard Genomic DNA

Table 1. *Salmonella* spp. used in this study

	Serovar	Serogroup		Serovar	Serogroup
1	*S. typhi* ATCC[a]19430	D1	30	*S. djugu*	C1
2	*S. paratyphi* ATCC11511	A	31	*S. meleagridis*	E1
3	*S. enteritidis* ATCC4931	D1	32	*S. hillingdon*	D2
4	*S. schottmulleri* ATCC10719	B	33	*S. istanbul*	C3
5	*S. choleraesuis* ATCC13312	C1	34	*S. lindenburg*	C2
6	*S. typhimurium* ATCC14028	B	35	*S. derby*	B
7	*S. gallinarum* ATCC9184	D1	36	*S. mbndaka*	C1
8	*S. london* ATCC8389	E1	37	*S. haardt*	C3
9	*S. enteritidis* ATCC13076	D1	38	*S. schwarzengrund*	B
10	*S. schwarzengrund* IVK B01177	B	39	*S. stanley*	B
11	*S. eingedi* IVK B01183	C1	40	*S. virchow*	C1
12	*S. uppsala*	B	41	*S. montevideo*	C1
13	*S. ohio*	C1	42	*S. weltevreden*	E1
14	*S. budapest*	B	43	*S. bareilly*	C1
15	*S. wien*	B	44	*S. rissen*	C1
16	*S. virginia*	C3	45	*S. senftenberg*	E4
17	*S. muenchen*	C2	46	*S. bardo*	C3
18	*S. cerro*	K	47	*S. anatum*	E1
19	*S. nigeria*	C1	48	*S. richmond*	C1
20	*S. blegdam*	D1	49	*S. uganda*	E1
21	*S. yeerongpilly*	E1	50	*S. ahmadi*	E4
22	*S. give*	E1	51	*S. dublin*	D1
23	*S. hadar*	C2	52	*S. hindmarsh*	C3
24	*S. essen*	B	53	*S. pakistan*	C3
25	*S. ruanda*		54	*S. eingedi*	C1
26	*S. nieukerk*	C4	55	*S. braenderup*	C1
27	*S. benin*	D2	56	*S. infantis*	C1
28	*S. sereman*	D1	57	*S. heidelberg*	B
29	*S. kentucky*	C3			

[a] ATCC: American Type Culture Collection, Manassas, VA

Table 2. Non-*Salmonella* strains used in this study

Species	Strains	Serotypes[a]	Isolation[b]
Listeria monocytogenes	ATCC[c]19113	3	human
Listeria monocytogenes	ATCC19114	4a	
Listeria monocytogenes	ATCC19115	4b	human
Listeria monocytogenes	ATCC19117	4d	sheep
Listeria monocytogenes	ATCC19118	4e	chiken
Listeria monocytogenes	HPB410	1/2a	
Listeria monocytogenes	ATCC35152		guinea pig
Listeria ivanivii	ATCC19119		sheep
Listeria innocua	ATCC33090	6a	cow brain
Listeria welshimeri	ATCC35897	6b	decaying plant
Listeria seeligeri	ATCC35967		soil
Listeria grayi	ATCC19120		chinchilla feces
Listeria murrayi	ATCC25401		corn stalks and leaves
Escherichia coli O26			
Escherichia coli O55			
Escherichia coli O111			
Escherichia coli O114			
Escherichia coli O119			
Escherichia coli O157:H7	ATCC43888		
Escherichia coli O157:H7	ATCC43894		
*Escherichia coli*K12	ATCC23736		
Shigella flexeri	ATCC9199		
Shigella sonnei	ATCC9120		
Yersinia enterocolitica	ATCC27729		

[a] Source: http://www.atcc.org/ except *L. monocytogenes* HPB410.
[b] Source: http://www.atcc.org/.

purification system were purchased from Promega (USA). The *Taq* DNA polymerase and reagents for the PCR and G-spin™ genomic DNA extraction kit were obtained from Intron Biotechnology (Korea). Ligase was obtained from Roche (Germany). Pork meat (for cutlet) was purchased at a supermarket (Samsung TESCO Home Plus, Gyeongju, Korea), and was tested before use as negative for the *Salmonella* with PCR. All chemicals, unless otherwise noted, were purchased from Sigma (USA).

Primers

The DNA primers used in this study are listed in Table 3. All primers were purchased from GenoTech (Daejeon, Korea). The *invA* gene (GenBank accession number AL513382) was chosen because this sequence was previously tested for detection of *Salmonella*[13,22,31-37].

Construction of the plasmid encoding *Salmonella invA* gene containing an 82- base deletion

All DNA manipulations were performed according to standard procedures[38]. The *Eco*RV site in the *invA* gene was created by overlap extension. The DG187/DG188 primer pair was used to amplifiy the DNA segment that contained the *Eco*RV site, together with upstream *Eco*RI sequences for subcloning. The DG189/DG190 primer pair was used to amplify the DNA segment that contained the *Eco*RV site together with downstream *Hin*dIII sequences for subcloning. The amplified DNAs were purified with a PCRquick-spin™ PCR Product Purification kit (Intron) and were used for amplification with the DG187/DG190 pair. The amplified DNA segment was cleaved with *Eco*RI/*Hin*dIII, and was cloned into the pGEM-4Z plasmid to get the pGEM-4Z

Table 3. Primers used in this study

Primer Name	Oligonucleotide sequence (5'-3')	Use
DG75	GAC CGC AAG GTT GAA ACT CA	Detection of *Listeria* 16S rDNA
DG76	CAG CCT ACA ATC CGA ACT GA	
DG129	TGG GAA ACT GCC TGA TGG AG	Detection of *Salmonella* 16S rDNA
DG130	ACC TTC CTC CCC GCT GAA AG	
DG131	GCT GGT CTG AGA GGA TGA CC	Detection of *E. coli*, *Yersinia*, and *Shigella* 16S rDNA
DG132	AAG GGC ACA ACC TCC AAG TC	
139	GTG AAA TTA TCG CCA CGT TCG GGC AA	Detection of *invA*
141	TCA TCG CAC CGT CAA AGG AAC C	
DG158	CGG ATC TCA TTA ATC AAC AAT A	Detection of *invA*
DG159	CTC TTT CGT CTG GCA TTA TC	
DG187	AAA TAC CGG CAG CGG GAA TTC CGG CAG AGT	Creation of *Eco*RV site in *invA*
DG188	GAT TTG AAG GCC GAT ATC ATT GAT GCG GAT	
DG189	ATC CGC ATC AAT GAT ATC GGC CTT CAA ATC	
DG190	CCT CAG TTT TTC AAG CTT TCC TGC GGT ACT	
DG215	AGG TTC AGA TCG CGA CGC GGA AGT	Creation of *Nru*I site in *invA*
DG216	ACT TCC GCG TCG CGA TCT GAA CCT	
DG217	TAT AGA ATA CGA ATT CCG GCA GAG T	
DG218	ATA GGG AGA CAA GCT TTC CTG CGG T	

invA RV. The *Nru*I site in the pGEM-4Z invA RV was also created by overlap extension. The DG217/DG215 primer pair was used to amplifiy the pGEM-4Z invA RV DNA segment that contained the *Nru*I site together with upstream *Eco*RI sequences for subcloning. The DG216/DG218 primer pair was used to amplify the pGEM-4Z invA RV DNA segment that contained the *Nru*I site downstream *Hin*dIII sequences for subcloning. The amplified DNAs were purified with the PCRquick-spin™ PCR Product Purification kit, and were used for amplification with the DG217/DG218 pair. The amplified DNA segment was cleaved with *Eco*RI/*Hin*dIII, and was cloned into the pGEM-4Z plasmid to get the pGEM-4Z invA RV/Nru. The pGEM-4Z invA RV/Nru was cleaved with *Eco*RV/*Nru*I, ligated, and introduced into *E. coli* DH5α. The colonies were screened for the absence of the *Eco*RV and *Nru*I sites by restriction enzyme digestion. After sequencing, the final clone, which had 82 bases deleted, was designated as pGEM-4Z invA ΔRV-NruI.

Preparation of DNA in artificially inoculated pork meat

The pork meat was homogenized with a Stomacher (AES Laboratoire, France) in distilled water (1:1, w/v) dispensed in bags, and stored at -70°C until use. The guanidine thiocyanate-phenol-chloroform method was used for the preparation of the PCR template[39]. Briefly, the artificially inoculated 0.1 g of pork meat was extracted with 0.25 mL of solution D (4M guanidine thiocyanate, 0.025M sodium citrate, 0.5% N-Lauroylsarcosine) and 0.5 mL of phenol-chloroform (1:1). The aqueous phase was extracted with 400 μL of chloroform and the DNA was precipitated with isopropanol and sodium acetate. The resulting pellet was washed once with 70% ethanol, dried, and used for PCR.

Polymerase chain reaction (PCR)

The PCR mixture contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM dNTPs, 2.5 units of *i-StarTaq*™ DNA polymerase, 100 pmol of each primer, and varying amounts of template DNA. The samples were denatured at 94°C for 5 min and subjected to amplification cycles in a thermocycler (My Cycler™ Thermal Cycler, BIO-RAD). Each cycle consisted of a 45-sec denaturation step (94°C), 45-sec annealing step (55°C), and 45-sec extension step (72°C). Finally, the products were extended for 7 min at the completion of 30 amplification cycles.

Results and Discussion

Detection of *Salmonella* spp.

Previously, Rahn et al.[22] reported that the detection sensitivity of the *invA* gene was approximately 300 cells per PCR reaction, and Malony et al.[40] reported 5-50 *Salmonella* colony forming units (cfu) per PCR reaction when they used the boiling method for *Salmonella* DNA extraction. Because the boiling method is usually applied to wash fluid from the surface of a food, it is not adequate to apply it to the direct extraction of *Salmonella* DNA from contaminated meat. Therefore, we decided to use other methods to extract DNA from the meat and compared the modified guanidine thiocyanate-phenol-chloroform method with two commercial kits (G-spin™ genomic DNA extraction kit and Wizard Genomic DNA purification system) for extracting the *Salmonella* DNA (Fig. 1). The modified guanidine thiocyanate-phenol-chloroform method (58-580 cfu) and G-spin™ genomic DNA extraction kit (14.6-146 cfu) were better than the Wizard Genomic DNA purification system (146-1,460 cfu) in detection sensitivity per PCR reaction with primers DG158/DG159 based on the *invA* gene. However, considering the sample size and difficulty in the application to pork meat, the following experiments were performed using the modified guanidine thiocyanate-phenol-chloroform method.

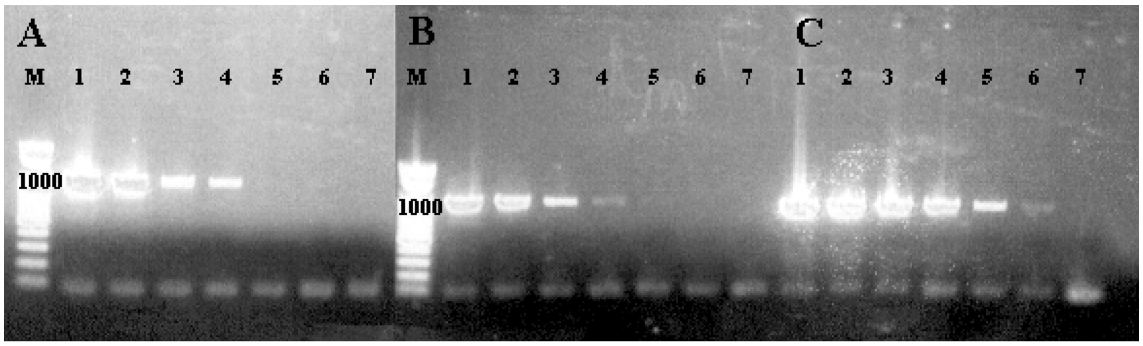

Fig. 1. Gel electrophoresis profile with three template DNA isolation methods. For PCR, the DG158/DG159 primer set was used and 1,031-bp PCR products were compared. The colony-forming units (cfu) of *S. typhimurium* ATCC14028 were determined by MPN. (A) Wizard Genomic DNA purification system (B) Modified guanidine thiocyanate-phenol-chloroform (C) G-spin™ Genomic DNA extraction kit (for bacteria). (A) and (C): Lane M, 100-bp DNA ladder; lane 1, 1.46×10^6 cfu; lane 2, 1.46×10^5 cfu; lane 3, 1.46×10^4 cfu; lane 4, 1.46×10^3 cfu; lane 5, 1.46×10^2 cfu; lane 6, 1.46×10 cfu; lane 7, 1.46 cfu (B): Lane M, 100-bp DNA ladder; lane 1, 0.58×10^6 cfu; lane 2, 0.58×10^5 cfu; lane 3, 0.58×10^4 cfu; lane 4, 0.58×10^3 cfu; lane 5, 0.58×10^2 cfu; lane 6, 0.58×10 cfu; lane 7, 0.58 cfu.

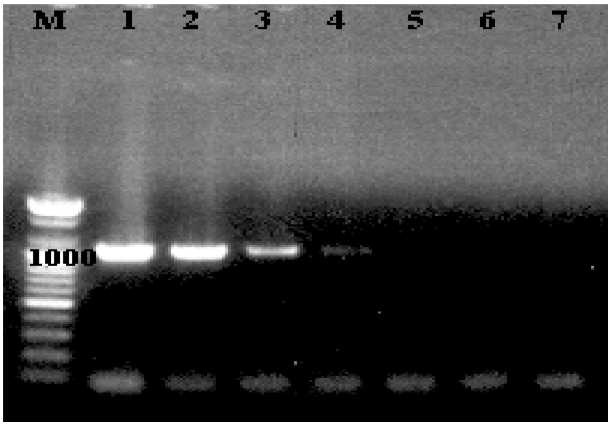

Fig. 2. Detection sensitivity of *Salmonella* in pork meat. *Salmonella* DNA was extracted using the modified guanidine-thiocyanate-phenol-chloroform method. The cfu of *S. typhimurium* ATCC14028 were determined by MPN. PCR products of 1,031-bp were detected from 1.46×10^6, 1.46×10^5, 1.46×10^4, and 1.46×10^3 cfu of *S. typhimurium* per 0.1 g of pork meat (lanes 1-4). No band was detected from 1.46×10^2, 1.46×10 and 1.46 cfu of *S. typhimurium* per 0.1 g of pork meat (lanes 5-7).

To measure the detection sensitivity in pork meat, homogenized pork meat (0.1 g) was artificially inoculated with $1.46\text{-}1.46\times10^6$ cfu of *Salmonella typhimurium* ATCC14028. The DNA was directly extracted with the modified guanidine thiocyanate-phenol-chloroform method and subjected to PCR amplification with primers DG158/DG159. The detection sensitivity was 146-1,460 cfu/0.1 g of pork meat (Fig. 2), which was similar to, or better than, that observed previously; that is, 10^3 cfu of *L. monocytogenes* in 0.5 mL of milk[26], 10^3 cfu of *Yersinia enterocolitica* in 0.5 mL of milk[28], 860 cfu of *L. monocytogenes* in 0.1 g of pork meat[29], 10^3 cfu *Salmonella* spp. per 1 mL milk[35] and 17,000 cfu *Salmonella typhimurium* per 0.15 g of wheat grain[34]. The cycle extension up to 50 cycles, or another 30 cycles of PCR, did not increase the sensitivity. After a 20-fold dilution with LB medium and incubation at 30ºC for 15 hr, improved sensitivity was achieved. Five cfu of *S. typhimurium* per 0.1 g of meat was easily detected (data not shown).

Rahn et al.[22] used the primer set 139/141 and amplified the 284-bp *Salmonella invA* specific DNA fragment. Recently, a *Salmonella* spp.-specific conventional PCR assay targeting the *invA* gene was validated in an international collaborative study, and was proposed as an international standard[40]. To evaluate the specificity, we tested 57 *Salmonella* strains comprised of 11 genogroups[39], and 24 non-*Salmonella* strains that included eight *E. coli* strains, 13 *Listeria* strains, two *Shigella* strains, and one *Yersinia enterocolitica*. The assay showed a 284-bp *Salmonella* specific band in all 57 *Salmonella* strains, but no false positive reaction occurred in the non-*Salmonella* strains tested (Fig. 3). However, *E. coli* O119 showed an approximate 450-bp non-specific PCR product (Fig. 3A lane 5). The band was consistent and strong. Rahn et al.[22] mentioned that they also observed this on at least one occasion, in 6 strains of *E. coli*, although they didn't mention the antigen type of *E. coli*, and stated that the non-specific product was obtained inconsistently and showed a weak band. Rahn et al.[22] also mentioned that *S. senftenberg* and *S. litchfield* gave false negative results, whereas Malony et al.[40] reported that *S. senftenberg* and *S. litchfield* gave positive bands when they were compared using the same primer set 139/141. Our repeated experiments clearly demonstrated that *S. senftenberg* produced a positive *Salmonella* specific 284-bp band (Fig. 3C, lane 45). However, we could not test *S. litchfield* due to the nonavailability of bacteria. At present, it is not clear why this different finding arose. One possible explanation might be due to differences in the PCR conditions between laboratories.

Competitive PCR for the direct enumeration of *Salmonella* spp. in pork meat

Previously we demonstrated that the amplification of radioisotope-labeled *hlyA* of *L. monocytogenes* sequences was still in the exponential range after 30 cycles under the PCR conditions of the study[26]. Therefore, we used the same conditions for cPCR. For the quantification of *Salmonella* spp., DNA from pork meat that had been artificially inoculated with 1.46×10^4 cfu of *S. typhimurium* ATCC14028 was co-amplified in the presence of known copy numbers of a competitor plasmid, pGEM-4Z invA ΔRV-NruI, which binds the same primers (primers 139 and 141) but has been deleted by 82-bp in order to distinguish it from natural sequences. The amplification efficiencies of the two different kinds of DNA should have been almost equal, because the same primers bind the same sequence for amplification. The only difference between the *invA* gene of the bacterial chromosome and the modified gene of pGEM-4Z invA ΔRV-NruI was the deleted sequences (82-bp). Since the relative amounts of the cPCR products generated from the amplification reflected the relative initial levels of the two different kinds of DNA, the cfu value of the *Salmonella* spp. was easily estimated by comparing the intensity of the two bands after electrophoresis. In fact, the calculated DNA copy number of the standard DNA and the cfu count deduced from the MPN were almost equal in this procedure using primers 139/141 (Fig. 4).

Several papers have described PCR detection of *Salmonella* after enrichment[13,19-21,32,34,35,41-44]; however, reports for direct enumeration of *Salmonella* in food or environmental samples using PCR are rare[34]. Even though some recent papers have described the automated detection and enumeration of *Salmonella* spp. in composted biosolids[36],

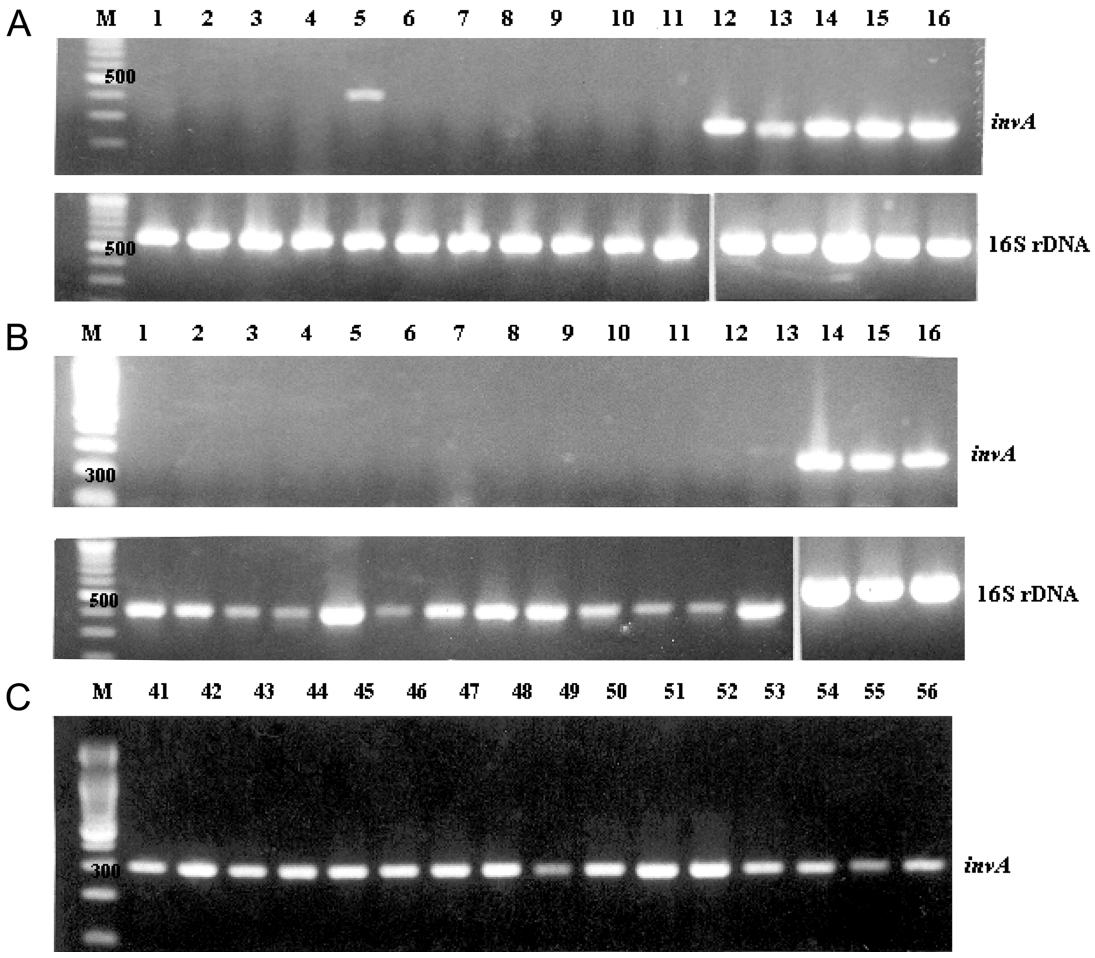

Fig. 3. Agarose gel electrophoresis of PCR products obtained from *E. coli, Shigella, Yersinia,* and *Salmonella* spp. For the detection of *invA*, the DG146/DG147 primer set was used and a 284-bp PCR product is shown. (A) For the detection of 16S rDNA, a DG131/DG132 primer set was used. Lane 1, *E. coli* O26; lane 2, *E. coli* O55; lane 3, *E. coli* O111; lane 4, *E. coli* O114; lane 5, *E. coli*, O119; lane 6, *E. coli* O157:H7 ATCC43888; lane 7, *E. coli* O157:H7 ATCC43894; lane 8, *E. coli* K12; lane 9, *Shigella flexeri* ATCC9199; lane 10, *Shigella sonnei* ATCC9120; lane 11, *Y. enterocolitica* ATCC27729; lane 12, *S. typhi* ATCC19430; lane 13, *S. paratyphi* ATCC11511; lane 14, *S. enteritidis* ATCC4391; lane 15, *S. schottmulleri* ATCC10719; lane 16, *S. choleraesuis* ATCC13312. (B) For the detection of the 16S rDNA of *Listeria* spp., the DG75/DG76 primer set was used. Lane 1, *L. mocytogenes* ATCC19113; lane 2, *L. mocytogenes* ATCC19114; lane 3, *L. monocytogenes* ATCC19115; lane 4, *L. monocytogenes* ATCC19117; lane 5, *L. monocytogenes* ATCC19118; lane 6, *L. monocytogenes* (HPB410); lane 7, *L. monocytogenes* ATCC35152; lane 8, *L. ivanovii* ATCC19119; lane 9, *L. innocua* ATCC33090; lane 10, *L. welshimeri* ATCC35897; lane 11, *L. seeligeri* ATCC35967; lane 12, *L. grayi* ATCC19120; lane 13, *L. murrayi* ATCC25401; lane 14, *S. typhimurium* ATCC14028; lane 15, *S. gallinarium* ATCC9184; lane 16, *S. london* ATCC8389. (C) The numbers on each lane are the *Salmonella* strain numbers given in Table 1.

waste water from municipal waste water treatment[37], meat[45], vegetable rinse-water[46], human feces[47], and environmental samples[35,48] using real-time PCR, the procedure still requires an expensive, special machine and reagents. Depending on the purpose, sometimes simple detection and rough estimation of the cfu are enough for routine control. The procedure described in this study does not provide accurate numbers of contaminated *Salmonella* spp. in pork meat. Nevertheless, it will be useful for rapid enumeration of *Salmonella* spp. under conditions when real-time PCR is not available.

Since the procedure described in this paper is basically PCR-based, it does not differentiate dead cells from viable ones and can thereby yield exaggerated results. A recent paper described the specific detection of viable *Salmonella* spp. using magnetic capture hybridization RT-PCR in seeded soil and chicken manure[49]. Experiments using RT-cPCR to determine viable *Salmonella* spp. in pork meat are ongoing.

In summary, we evaluated that the *invA* gene is unique to *Salmonella* spp., and have shown that primers based on this region can be used to differentiate *Salmonella* and other organisms. We cloned and modified the *invA* gene and

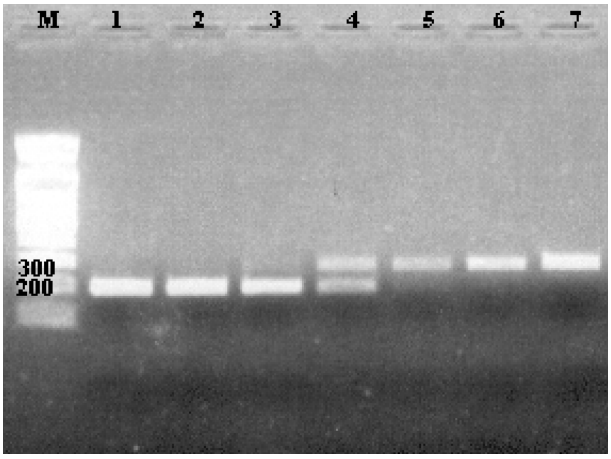

Fig. 4. Agarose gel electrophoresis after cPCR. The upper band (284-bp) was derived from artificially inoculated *S. typhimurium* (ATCC14028) DNA and the lower band (202-bp) was derived from pGEM-4Z invA ΔRV-NruI standard DNA. 1.46×10^4 cfu of *S. typhimurium* were artificially inoculated in 0.1g of pork meat. Lane M, 100-bp DNA ladder as a size marker; lane 1, 1.46×10^7 copies of standard DNA (pGEM-4Z invA ΔRV-NruI); lane 2, 1.46×10^6 copies of standard DNA; lane 3, 1.46×10^5 copies of standard DNA; lane 4, 1.46×10^4 copies of standard DNA; lane 5, 1.46×10^3 copies of standard DNA; lane 6, 1.46×10^2 copies of standard DNA; lane 7, 1.46×10 copies of standard DNA.

successfully used it with cPCR for the rapid enumeration of *Salmonella* spp. in contaminated pork meat. The results of this study provide the basis for conventional PCR as a rapid, inexpensive, and convenient method for the detection and enumeration of *Salmonella* spp. in a practical field such as HACCP.

요 약

돼지고기에 오염된 살모넬라의 수를 직접 측정하는 방법으로 경쟁적 PCR(competitive PCR)을 사용하였다. 세 가지 DNA추출방법을 비교한 후 guanidine thiocyanate-phenol-chloroform 방법을 일부 수정하여 인위적으로 살모넬라를 오염시킨 돼지고기로부터 DNA를 직접 추출하는 방법으로 선택하였다. Rahn 등(Mol. Cell. Probes 1992년)이 이미 보고한 284-bp *invA* gene의 특이성을 평가하기 위해 살모넬라 57균주와 살모넬라가 아닌 균주 24개를 사용하였다. 시험해 본 모든 살모넬라 균주는 *invA* 양성으로 살모넬라가 아닌 모든 균주는 *invA* 음성으로 판명되었으며 살모넬라가 아닌 균주 중 양성으로 잘 못 판명된 경우는 없었다. 돼지고기 0.1 g을 사용할 경우 검출한계는 1,460 cfu였다. 경쟁적 PCR을 위해 *invA* gene 중 82-bp를 결실시킨 DNA조각을 pGEM-4Z백터에 클로닝을 하였다. 인위적으로 오염된 돼지고기로부터 추출한 살모넬라 염색체 DNA와 이와 같은 primer 결합자리를 가진 경쟁 DNA를 동시에 경쟁적 PCR로 증폭하였다. 경쟁적 PCR을 사용하여 결정한 균수와 MPN방법으로 결정한 균수는 거의 유사하였으며 전체 과정을 수행하는 데 약 5시간이 소요되었다.

Acknowledgements

This study was supported by the Dongguk University. We would like to thank Hyojung Lee and Hyungkun Lim for their technical assistance.

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