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Toxin Gene Typing, DNA Fingerprinting, and Antibiogram of *Clostridium perfringens* Isolated from Livestock Products

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Abstract

Forty *Clostridium perfringens* isolates were obtained from twelve animal products, following the examination of eighty six beef, pork, broiler chicken and salami meat products, and eleven milk powder products. There were 21 isolates from salami stored at 25℃, 3 isolates from pork, 4 isolates from beef, 9 isolates from broiler chicken, and 3 isolates from milk powder. Only the *cpa* gene encoding a toxin among the 5 toxin genes tested (*cpa*, *cpb*, *etx*, *iap*, and *cpe*) was detected in all forty isolates, suggesting contamination with *C. perfringens* type A. DNA fingerprinting analysis using PCR of the tRNA intergenic spacer (tDNA-PCR) and the 16S-23S internal transcribed spacer (ITS-PCR), and randomly amplified polymorphic DNA (RAPD) analysis were attempted to differentiate the isolates. RAPD analysis was the most discriminating method among the three PCR analyses. Isolates from the same products tended to show similar RAPD patterns. Antimicrobial susceptibility tests showed that some isolates from broiler chickens had the same antibiogram with multiple resistance to streptomycin, colistin, and ciprofloxacin. Antibiograms were similar between isolates from the same livestock products, but differed considerably between the products.

Key words : *Clostridium perfringens*, toxin gene, DNA fingerprinting, antibiogram, livestock product, food

INTRODUCTION

Clostridium perfringens strains are pathogenic bacteria ubiquitous in nature. They are Gram-positive rod in microscopic observation and form spore in the certain stage of vegetative growth (Shimizu *et al.*, 2002). They are frequently found in soil, rubbish, and intestine of human and animals and involved in food poisoning, necrotic enteritis, and intestinal intoxication (Petit *et al.*, 1999; Rood, 1998).

C. perfringens strains were classified into five types of A, B, C, D, and E based on production of virulent toxins (Sterne and Warrack, 1964). All strains of the five types produce α toxin (McDonel, 1986). The strains of type B and C additionally turn out to be β toxin which is associated with intestinal necrosis. The β toxin is encoded by *cpb* gene located in plasmid. The strains of type B and D also secrete ε toxin which is encoded by *etx* gene in plasmid (Hunter *et al.*, 1993). Type E strain contains ι toxin in addition to a toxin (Stern and Warrack, 1964). Recently β_2 toxin was detected in *C. perfringens* strains which were isolated from horse and lamb which had intestinal disorder (Herholz *et al.*, 1999).

Enterotoxin produced by toxigenic *C. perfringens* during sporgenesis was reported to cause food poisoning in human (Hobbs, 1965; Saito *et al.*, 1992; Todd, 1978). Food poisoning may also occur when food containing the bacteria at the level of $10^8 \sim 10^9$ is ingested, but the symptom is mild (Richardson and Granum, 1985). Isolates with *C. perfringens enterotoxin*(*cpe*) *gene* were detected in approximately 1.4% of 900 American retail foods not associated with food poisoning outbreak (Wen and McClane, 2004). A DNA fragment of 364 bp containing *cpe* gene was mutiplicated from plasmid by using PCR (Jung, 2000).

Food poisoning outbreaks, which were observed more frequently than any other diseases caused by *C. perfringens*, occurred when meat and meat products containing more than 10^6 cell per gram were ingested. The incriminated foods were meat, processed meat products, and foods fried in oil. The

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foods, such as beef stews which were cooked in bulk and cooled slowly at room temperature were frequently associated with food poisoning outbreak, since the heat-resistant spores of *C. perfringens* survived cooking and multiplied in anaerobic condition of food out of which oxygen was removed during heating (Jung *et al.*, 1998).

C. perfringens is associated with intestinal disorder, such as necrotic enteritis and enterotoxaemia, and haemorrhagic diarrhoea in farm animals. Kim *et al.* (1997) determined toxin types of 30 *C. perfringens* isolates from healthy and diseased chickens with necrotic enteritis using toxin neutralization assay. Among them are 26 type A isolates, 2 type C isolates, and 2 unclassifiable isolates. Jung (2000) reported that all 30 *C. perfringens* isolates from chicken infected with necrotic enteritis were type A. Among 21 *C. perfringens* isolates from pigs with enterotoxaemia were 17 type A isolates, 2 type B isolates, and 2 type C isolates.

Immunological toxin typing methods include reversed passive latex agglutination assay (Jung, 1997) and enzyme-linked immunosorbent assay (McClane and Strouse, 1984) and molecular genetical toxin typing methods include DNA hybridization using specific probe (Daube *et al.*, 1996) and PCR analysis (Fach and Guillou, 1993; Kanakaraj *et al.*, 1998; Miwa *et al.*, 1998).

In molecular epidemiological studies of bacteria, pulsed field gel electrophoresis analysis is the primary method. Alternative DNA fingerprinting includes ribotyping and PCR-based analyses, such as randomly amplified polymorphic DNA (RAPD) analysis and repetitive element-based PCR(rep-PCR). Antibiogram (antibiotic susceptibility profiles) is also used to differentiate bacterial strains(Versalovic and Lupski, 2002; Kilic *et al.*, 2002; Schalch *et al.*, 2003). The objectives of this study were to determine the toxin types of *C. perfringens* isolates from beef, pork, broiler chicken, salami, and milk powder sold in Korean market and to evaluate capacity of PCR-based DNA fingerprinting analysis and antibiogram in differentiating the isolates.

MATERIALS AND METHODS

Livestock Products

Beef, pork, broiler chicken, salami, and milk powder were purchased from local market in Wonju, Korea. Salami was stored at 25℃ for 64 days before sampling for experiments.

Reference Strains of *C. perfringens*

C. perfringens ATCC 12917 (type A) was purchased from American Type Culture Collection. *C. perfringens* KCCM 40947 (type B) was purchased from Korean Culture Center of Microorganism. *C. perfringens* type C, type D, and type E were obtained from National Veterinary Research and Quarantine Service in Korea.

Isolation and Identification of *C. perfringens*

Twenty five gram food sample was added to 225 mL cooked meat medium (Oxoid) and the inoculated medium was blended in a stomacher and then incubated anaerobically at 35 ℃ for 18~24 hr. A loopful culture of the medium was streaked on tryptose sulphite cycloserine agar (Oxoid) and the agar was incubated anaerobically at 35℃ for 18~24 hr. The black colony was transferred to thioglycollate medium (Oxoid) and reinforced clostridial agar (Oxoid). The culture was subjected to Gram staining, iron-milk medium test, motility-nitrate medium test, and lactose-gelatin medium test for identification. Sporulation medium was used for spore staining.

DNA Isolation

Reference strains and isolates from livestock products were inoculated into reinforced clostridial agar and incubated anaerobically at 35℃ for 18~24 hour. The colony on the agar was scraped and dispersed into 0.9% saline solution. The turbidity of the bacterial suspension was adjusted to make 12×10^8 cell/mL using McFarland nephelometer. 83 μL suspension was centrifuged at 5,000 × g for 5 min. The resulting pellet was resuspended in 200 μL Chelex resin(50 g/mL: Bio-Rad) and 4 mL of proteinase K (10 mg/mL: Sigma) and incubated at 56℃ for 30 min. Finally, the mixture was incubated at 100℃ for 8 min and centrifuged. The resulting supernatant was saved and frozen at −20℃ for later use.

Toxin Gene Typing

The PCR primers (Table 2; Buogo *et al.*, 1995; Braun *et al.*, 2000) for *cpa*, *cpb*, *iap*, *etx*, and *cpe* genes were used to determine a type of *C. perfringens* toxin gene(Table 1). Four pmole primer and 5 μL DNA were added into PCR Premix (Bioneer) containing 1U Taq DNA polymerase, 5 nmol NTP, 200 nmol Tris-HCl (pH 9.0), and 800 nmole KCl, and 30 nmole $MgCl_2$. Distilled water was then added to make up to

Table 1. Nucleotide sequences of the PCR primers used for toxin gene typing of *C. perfringens*

Toxin gene	Primer nucleotide sequence[1,2]	DNA product (bp)	Annealing temperature (℃)
α toxin *cpa*	L:5' AAGATTTGTAAGGCGCTT 3' R:5' ATTTCCTGAAATCCACTC 3'	1,167	46
β toxin *cpb*	L:5' AGGAGGTTTTTTTATGAAG 3' R:5'TCTAAATAGCTGTTACTTTGTG 3'	1,025	39
ε toxin *etx*	L:5' AAGTTTAGCAATCGCATC 3' R:5' TATTCCTGGTGCCTTAATT 3'	961	48
Enterotoxin *cpe*	L:5' TAACAATTTAAATCCAATGG 3' R:5' ATTGAATAAGGGTAATTTCC 3'	933	46
ι toxin *iap*	L:5' AATGCCATATCAAAAAATAA 3' R:5' TTAGCAAATGCACTCATATT 3'	821	52

[1] Buogo *et al.*(1995).
[2] Braun *et al.*(2000).

Table 2. Antibiograms of reference strains of *C. perfringens*

Strains and isolates	Antibiotics									
	Amp	Amo	Str	Gen	Neo	Cep	Ery	Oxy	Col	Cip
ATCC 12917	++	+++	+	+	+	++	++	++	−	++
KCCM 40947	+	++	−	+	+	++	+	++	−	+
Type C	+++	+++	+++	+++	+++	+++	++	++	−	++
Type D	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Type E	+++	+++	+++	+++	+	++	+++	+++	−	++
SP1	+	++	−	+	+	++	+	++	−	+
ST1	+++	+++	+++	+++	+	++	++	++	+++	++
ST2	+++	+++	+++	+++	+++	+++	++	+	−	++
ST8	+++	+++	+++	+++	+++	+++	++	+	−	++
SH5	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
SJ2	+++	++	+++	+	+	++	+	+	−	++
SJ3	+++	+++	+++	+	+	++	+	+	−	++
BE3	++	++	−	+	+	+	+	+	+	++
CP3	++	++	−	+	+	+	+	++	+	++
PO3	++	++	−	+	+	+	+	++	−	++
PQ1	++	++	−	+	+	+	+	++	−	++
CH2	+++	+++	+++	+	+	++	++	+	−	−
CH3	+++	+++	−	+	+	++	++	+	−	−
CM1	+++	++	−	+	+	++	++	+	−	−
CM5	++	++	−	+	+	++	++	+	−	−
MG3	+++	++	−	+	+	++	++	++	−	++
MG4	+++	++	−	+	+	++	++	++	−	++

Amp: ampicillin, Amo: amoxicillin, Str: streptomycin, Gen: gentamycin, Neo: neomycin, Cep: cephalomycin, Ery: erythromycin, Oxy: oxytetracycline, Col: colistin, Cip: ciprofloxacin.
−: no inhibition zone, +: 〈2mm inhibition zone diameter, ++: 2~5mm inhibition zone diameter, +++: 〉 5 mm inhibition zone diameter.

20 μL. The PCR condition includes pre-denaturation step at 94℃ for 7 min and then 30 cycles of denaturation step at 94 ℃ for 30 sec, annealing step at the temperature specified in Table 2 for 30 sec, and extension step at 72℃ for 30 sec. Final extension step at 72℃ for 4 min was included. The amplified DNA was separated in 0.5% agarose gel electrophoresis at 50 V.

tDNA- PCR and ITS-PCR Analysis

The primers for tDNA-PCR were T5A (5'-AGTCCGGTGC TCTAACCAACTG AG-3') and T3B (5'-AGGTCGCGGGTC GAATCC-3'). The primers for ITS-PCR was L1 (5'-caa-ggcatc caccgt-3') and G1 (5'-gaagtcgtaacaagg-3'). Ten pmole primers and 5 μL DNA were added into PCR Premix (Bio-

neer). Distilled water was then added to make up to 20 μL. The PCR condition includes pre-denaturation step at 94℃ for 7 min and then 30 cycles of denaturation step at 94℃ for 30 sec, annealing step at 50℃ for 30 sec, and extension step at 72℃ for 1 min. Final extension step at 72℃ for 10 min was included. The amplified DNA was separated in 2% agarose gel electrophoresis.

RAPD Analysis

Ten OPC primers (01, 04, 05, 06, 08, 09, 12, 13, 17, 19: Operon) were used for RAPD analysis. Four pmole primer and 30 nmole $MgCl_2$ were added to PCR Premix(Bioneer). Distilled water was added to make up to 20 μL. The PCR condition includes pre-denaturation step at 94℃ for 7 min and then 45 cycles of denaturation step at 94℃ for 1 min, annealing step at 37℃ for 1 min, and extension step at 72℃ for 1 min. Final extension step at 72℃ for 4 min was included. The amplified DNA was separated in 2% agarose gel electrophoresis.

Antibiotic Susceptibility Test

The colony on reinforced clostridial agar incubated at 35℃ for 24 hr was transferred to saline solution. The suspension volume was adjusted to make 0.5 McFarland nephelometer. Reinforced clostridial agar(15 mL) was added with 0.1 mL suspension. Four antibiotic discs (BBL Sensi-Disc, Becton, Dickinson, Co) were placed on each agar plate. The agar was incubated anaerobically at 35℃ for 24 hr. The diameter of inhibition zone was measured. Each disc contained 10 μg gentamycin, 10 μg colistin, 30 μg neomycin, 10 μg ampicillin, 10 μg streptomycin, 20 μg amoxicillin, 5 μg ciprofloxacin, 30 μg oxytetracycline, 15 μg erythromycin, and 30 μg cephalosporin.

RESULTS AND DISCUSSION

Forty *C. perfringens* isolates were obtained from 12 products of meat, meat products and milk powder after 86 meat and meat products and 11 milk powder products were examined in this study. They were 21 isolates from salami, 3 isolates from pork, 9 isolates from broiler chicken, 4 isolates from beef, and 4 isolates from milk powder. They were given codes which consisted of capital letters and numbers. The isolates with same capital letter were from a same animal product. SJ1, SJ2, and SJ3 were from a salami product without storage. SP1, SP5, and SH5 were isolated from two different salami products stored at 25℃ for 21 day. ST1, ST2, ST3, ST4, ST5, ST6, ST7, ST7, ST7, ST8, ST9, ST10, ST11, ST12, ST13, ST14, and ST15 were isolated from a salami product stored at 25℃ for 64 day. BE1, BE2, BE3, and BF2 were from two different beefs. PO3, PP1, and PQ1 were from three different porks. CP3, CH1, CH2, CH3, CH4, CM1, CM3, CM4, and CM5 were from three different chickens. MG2, MG3, and MG4 were from a milk powder product.

The toxin gene types of *C. perfringens* reference strains (ATCC 12917, KCCM 40947, Type C, Type D, and Type E) were examined using PCR analysis with toxin gene specific primers(Table 1, Fig. 1). All five reference strains showed 1167 bp DNA of *cpa* gene encoding α-toxin. KCCM 40947 and Type C strain showed 1025 bp DNA of *cpb* gene encoding β-toxin. KCCM 40947 and Type D strains showed 961bp DNA of *etx* gene encoding ε-toxin. Type E strain showed 821 bp DNA of *iap* gene encoding ι toxin. ATCC 12917 strain showed 933 bp DNA of *cpe* gene encoding enterotoxin. The toxin gene typing results confirmed the known toxin types of the reference strains. All 40 isolates from animal products used in this study showed 1167 bp DNA of *cpa* gene but not the other toxin genes, concluding that they were *C. perfringens* type A (Fig. 2).

Park *et al.* (1996) reported in their study employing multiplex PCR that all *C. perfringens* isolates from chicken with necrotic enteritis were type A producing only α toxin. Toxin neutralization assay (Jung, 1998) showed that all 36 *C. perfringens* isolates from chicken with necrotic enteritis were

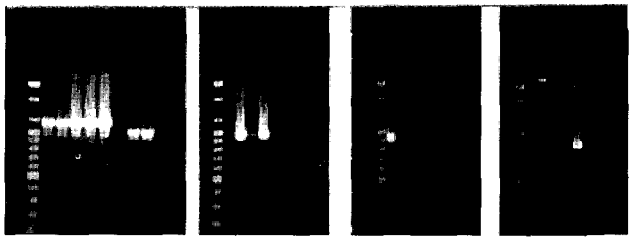

Fig. 1. Toxin gene typing of reference strains of *Clostridium perfringens* in agarose gel electrophoresis. A: *cpa*(1167 bp) and *cpb*(1025bp), B: *etx*(961bp), C: *cpe*(933bp), D: *iap*(821bp), Lanes: M, 100bp molecular size marker (Bioneer); 1, ATCC 12917 2, KCCM 40947 3, Type C 4, Type D ; 5, Type E.

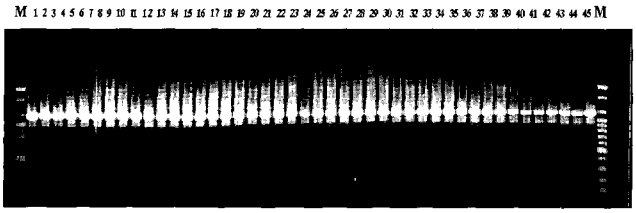

Fig. 2. PCR products (1,167 bp) of *cpa* gene obtained from reference strains and isolates of *C. perfringens* in agarose gel electrophoresis. Lanes: M, 100bp molecular size marker (Bioneer); 1, ATCC 12917 2, KCCM 40947 3, Type C 4, Type D; 5, Type E; 6, SP1; 7, SP5; 8, ST1; 9, ST2; 10, ST3; 11, ST4; 12, ST5; 13, ST6; 14, ST7; 15, ST8; 16, ST9; 17, ST10; 18, ST11; 19, ST12; 20, ST13; 21, ST14; 22, ST15; 23,SH5; 24, SJ1; 25, SJ2; 26, SJ3; 27, BE1; 28, BE2; 29, BE3; 30, CP3; 31, PO3; 32, BF2; 33, PP1; 34, PQ1; 35, CH1; 36, CH2; 37, CH3; 38, CH4; 39, CM1; 40, CM3; 41, CM4; 42, CM5; 43, MG2; 44, MG3; 45, MG4.

type A. All 17 isolates from cattle with enterotoxaemia were type A. However, among 21 isolates from pig with enterotoxaemia, type A, type B, and type C were 17(81%), 2(9.5%), and 2(9.5%), respectively.

The enterotoxin-positive *C. perfringens* type A is an important pathogen to cause intestinal infection in human and animal. 2~5% of type A strains were reported to possess *cpe* gene encoding enterotoxin of 35 kDa(Daube *et al.*, 1996; McClane, 2001; Songer, 1996). According to McDonel (1986), all types of *C. perfringens* had *cpa* gene encoding α toxin. Sparks *et al.* (2001) reported that 8% pigs infected with enterotoxaemia harbored *cpe* gene-positive *C. perfringens*. Kanakaraj *et al.* (1998) showed that all 97 *C. perfringens* isolates from healthy pigs were *cpe* gene-negative. Saito (1990) reported that only 2% of *C. perfringens* isolated from meat and fish were *cpe* gene-positive. Tschirdewahn *et al.* (1991) reported that the percentage of *cpe* gene-positive isolates from feces of cattle, chicken, and pig were 22%, 10%, and 0%, respectively. Nested PCR analysis by Miwa *et al.* (1998) showed that 37% of chicken contained *C. perfringens*. Among them. 17% was *cpe* gene-positive. PCR analyses of *cpa* gene and *cpe* gene in *C. perfringens* showed that 40 isolates from 131 foods sold in USA market contained *cpa* gene but not *cpe* gene(Lin and Labbe, 2003). Our study also showed that all 40 isolates from meat, salami, and milk powder were *cpe*-negative *C. perfringens* type A.

Diversity and genetical relationship of 40 *C. perfringens* isolates were assessed in tDNA-PCR, ITS-PCR, and RAPD

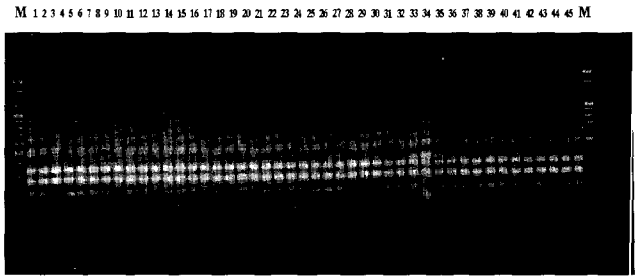

Fig. 3. tDNA-PCR analysis of reference strains and isolates of *C. perfringens* in agarose gel electrophoresis. Lanes: M, 100bp molecular size marker(Bioneer); 1~45, same as in Fig. 2.

analysis. In agarose electrophoresis of tDNA-PCR, the reference strains and 37 isolates, except PO3 and PP1 from pork and BF2 from beef, showed a same DNA band pattern consisting of 750bp, 500bp, 330bp, 250bp, 180bp, and 90bp (Fig. 3). In agarose electrophoresis of ITS-PCR, All reference strain and isolates showed similar DNA patterns having two major DNA bands of 520 bp and 370bp (Fig. 4).

tDNA-PCR amplifies the intergenic spacer between tRNA gene and ITS-PCR amplifies internal transcribed spacer(ITS) between 16S rRNA gene and 23S rRNA gene. Since these genes are multi-copy genes, the diversity of the spacer regions was used to differentiate between genera and species of bacteria. This study showed that tDNA-PCR and ITS-PCR were not appropriate to determine genetical relationship between reference strains and isolates of *C. perfringens*. But PCR analysis of tRNA intergenic spacer (tDNA-PCR) and of the 16S-23S internal transcribed spacer (ITS-PCR) and random amplified polymorphic DNA (RAPD) analysis were evaluated for their usefulness in characterization of *Enterobacter cloacae* isolates by Clementino *et al.* (2001).

RAPD analysis was used to evaluate diversity of the isolates. The OPC-6 primer produced multiple discrete DNA

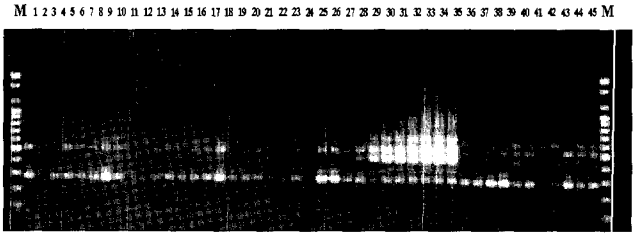

Fig. 4. ITS-PCR analysis of reference strains and isolates of *C. perfringens* in agarose gel electrophoresis. Lanes: M, 100bp Molecular Size Marker(Bioneer); 1~45, same as in Fig. 2.

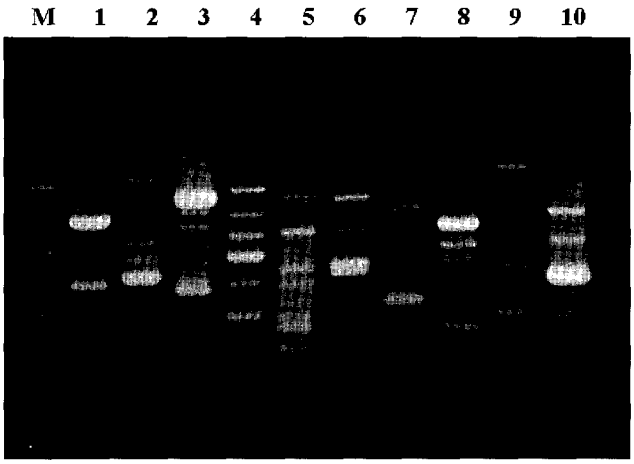

Fig. 5. RAPD analysis of *Clostridium perfringens* KCCM 40947 using ten OPC primers(Operon) in agarose gel electrophoresis. Lanes: M, 100bp molecular size marker(Bioneer); 1, OPC 01 2, OPC 04; 3, OPC 05; 4, OPC 06 5, OPC 08 6, OPC 09 7, OPC 12 8, OPC 13 9, OPC 17 10, OPC 19.

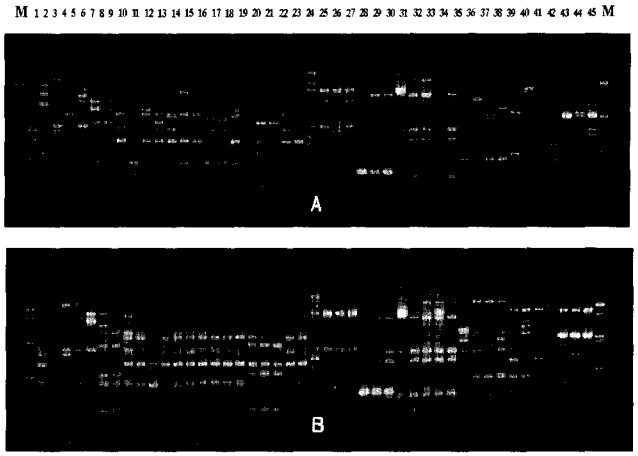

Fig. 6. RAPD analysis using OPC-6 primer of reference strains and isolates of *C. perfringens* by agarose gel electrophoresis. Lanes: M, 100bp molecular size marker (Bioneer); 1, ATCC 12917 2, KCCM 40947 3, Type C 4, Type D; 5, Type E; 6, SP1; 7, SP5; 8, ST1; 9, ST2; 10, ST3; 11, ST4; 12, ST5; 13, ST6; 14, ST7; 15, ST8; 16, ST9; 17, ST10; 18, ST11; 19, ST12; 20, ST13; 21, ST14; 22, ST15; 23, SH5; 24, SJ1; 25, SJ2; 26, SJ3; 27, BE1; 28, BE2; 29, BE3; 30, CP3; 31, PO3; 32, BF2; 33, PP1; 34, PQ1; 35, CH1; 36, CH2; 37, CH3; 38, CH4; 39, CM1; 40, CM3; 41, CM4; 42, CM5; 43, MG2; 44, MG3; 45, MG4.

bands (Fig. 5). RAPD analyses using OPC-6 of the reference strains and isolates were duplicated to compensate its innate lack of repeatability and missing lanes (Fig. 6, A and B). The five reference strains showed different DNA band patterns. The DNA band patterns in Fig. 6 B showed more similarity between isolates from same products than those in Fig. 6 A. The DNA band patterns of the isolates were considerably differed between the products from which they were isolated. Some of the isolates from a same food, such as ST1, ST2, ST5, ST6, and ST7 from salami stored at 25℃ for 64 day, SP1 and SP5 from salami stored at 25℃ for 21 day, and BE2 and BE3 from beef showed closely similar DNA patterns, respectively. The isolates from porks, such as PO3, PP1, and PQ1, showed relatively similar DNA patterns. However, the isolates from broiler chickens, such as CP3, CH1, CH2, CH3, CH4, CM1, CM3, CM4, and CM5, showed relatively heterogenous DNA patterns.

Since RAPD analysis can be done without sophisticated instrument used in PFGE analysis and showed variable DNA patterns among bacterial isolates, it is used frequently to determine genetical relationship between bacteria. But due to its lack of reliability and repeatability, caution should be exercised in interpretation of the data. However, *C. perfringens* isolates in this study could be differentiated by using RAPD analysis. The fact that the isolates from same products showed similar patterns with more common bands indicated that *C.perfringens* strains in the same products tended to be genetically homogenous. But problems of RAPD analysis were variability from day to day or from lab to lab in patterns and unreliability within the same experiment on the same day (Meunier and Grimont, 1993).

Antibiotic susceptibility assay performed to characterize relationships between the reference strains and some isolates which were selected based on different DNA patterns in RAPD analysis and to assess their resistance to antibiotics (Table 2). The reference strains except type D were resistant to colistin and only KCCM 40947 was resistant to streptomycin. The percentages of the isolates resistant to colistin, streptomycin, and ciprofloxan were 76%, 59%, and 18%, respectively. The isolates of CH2, CH3, CM1, and CM5 from broiler chickens showed a same antibiogram with multiple resistance to streptomycin, colistin, and ciprofloxacin. Ampicillin, amoxicillin, gentamycin, neomycin, cephalomycin, erythromycin, and oxytetracyclin were active in inhibiting growth of *C. perfringens*.

The isolates from same products, such as ST2 and ST8 from salami stored at 25℃ for 64 day, SJ2 and SJ3 from salami without storage, and MG3 and MG4 from milk powder product showed same antibiogram, respectively. However, the antibiograms of the isolates considerably differed between

different livestock products.

The multiple antibiotic resistances of some isolates from broiler chickens in this study may suggest that antibiotics are intensively used in broiler chicken operations. Antibiotic susceptibility of *C. perfringens* isolates from broiler chickens was reported by Devriese *et al.* (1993), Martel *et al.*, (2004), and Watkins *et al.*, (1997). Antibiotics used for growth promotion of broiler chicken may suppress intestinal colonization of susceptible *C. perfringens* strains and prevent pathogenic effects. The fact that the antibiograms of the isolates differ considerably between the livestock products suggested that usage of antibiotics as feed additives and veterinary drug during livestock production should influences antibiotic resistance of intestinal microorganism, *C. perfringens*.

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