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Analysis of Major Foodborne Pathogens in Various Foods in Korea

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Abstract Foodborne pathogenic bacteria in various food samples in Korea were monitored and the obtained data was statistically analyzed. A total of 1,240 food samples including 280 *sashimi*, 244 processed frozen products, 258 *kimbab* (cooked rice wrapped with seaweed), 337 soybean pastes were obtained from 7 cities including Seoul in Korea. Microorganisms tested were *Bacillus cereus*, *Salmonella* spp., *Staphylococcus aureus*, *Escherichia coli*, *E. coli* O157:H7, *Vibrio parahaemolyticus*, *Yersinia enterocolitica*, *Listeria monocytogenes*, *Campylobacter jejuni*, and *Clostridium perfringens*. The contaminated microorganisms in food samples were comprised of 10.55% *B. cereus*, 2.7% *S. aureus*, 2.0% *V. parahaemolyticus*, 0.8% *C. perfringens*, 0.2% *Y. enterocolitica*, and 0.1% of *L. monocytogenes*, respectively. *Salmonella* spp., *C. jejuni*, and *E. coli* O157:H7 were not detected in any of the food samples. Particularly, *B. cereus* that harbors the enterotoxin gene was detected in various foods and regions in Korea, therefore it should be a given special consideration not to allow the hazardous level of contamination.

Keywords: foodborne pathogenic microorganism, monitoring, polymerase chain reaction (PCR), toxin

Introduction

Even though there has been significant development in the food industry and food safety and management, food poisoning incidents have continuously increased in Korea. In particular, the number of patients per incident of food poisoning has dramatically increased over the last several years, according to the data from the report of status and precautions for outbreaks of food poisoning published by the Korea Food & Drug Administration (1). It represents that the incidents of food poisoning become bigger. Thus, outbreaks of food poisoning are one of the major problems in terms of social and economic losses.

In the past, food poisoning incidences mainly occurred between the months of May and September. However, recently, food poisoning has occurred in all seasons due to environmental changes such as global warming and the elevation of room temperature. Also, the causative pathogens of food poisoning have become varied from mainly *Salmonella* spp., *Staphylococcus aureus*, and *Vibrio parahaemolyticus* to other pathogens such as *Clostridium perfringens*, *Bacillus cereus*, *Escherichia coli* 0157:H7, and *Listeria*, and Norovirus. This may be because of a trend towards westernized eating habits and increased catering services.

In this paper, the contamination status for 10 major foodborne pathogens in distributed food samples, including ready-to-eat food, collected from 7 cities in Korea was investigated to provide information for the prevention of food poisoning and to collect systematic data for all foodborne pathogens. In turn, this information could be applied to establish risk management strategies against microorganisms and for the further development of food safety and sanitation policies.

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Materials and Methods

Samples and materials The samples included 337 samples of fermented soybean source, 258 *kimbab* (cooked rice wrapped with sea weed) and lunch boxes, 280 *sashimi*, 244 frozen and processed meat products, and 121 samples of regionally specialized foods (some foods that are famous in the specific regions in Korea such as seafood from Busan, tea leaves from Gwangju, fresh fruit juices from Daejeon, ready-to-eat side dishes from Daegu). In total 1,240 samples were obtained and examined by qualitative and quantitative analysis for *Salmonella* spp., *S. aureus*, *V. parahaemolyticus*, *Y. enterocolitica*, *C. jejuni*, *C. perfringens*, *E. coli*, *E. coli* 0157:H7, *B. cereus*, and *L. monocytogenes*. Isolates from these foods were tested for identification and characterization and for information of their pathogenicity.

Isolation and identification of *Salmonella* spp. A quantity (225 mL) of selenite broth (Difco Lab., Detroit, MI, USA) was added to 25 g of the sample and was mixed thoroughly. It was then incubated at 37°C for 18-24 hr and 100 μL of the mixture was extracted and inoculated into the Rappaport-Vassiliadis broth as an enrichment media (Merck, Damstradt, Germany) which was then again incubated at 43°C for 18-24 hr. It was inoculated onto the selective medium, xylose-lisine-deoxycholate agar (XLD, Difco Lab.), and incubated for 18-24 hr at 37°C. After incubation, a typical colony was selected and tested for

identification through triple sugar iron agar (Difco Lab.), API 20E (bioMerieux, Marcy l'Etoile, France), and the cohesive reaction of the serum.

S. aureus/Isolation and identification: A quantity (225 mL) of tryptic soy broth (TSB, Difco Lab.) containing 10% NaCl was mixed with the 25 g of the sample and enriched for 18-24 hr at 37°C. After the enrichment, it was inoculated on the egg yolk added mannitol salt agar (MSA, Difco Lab.) and incubated for 24-48 hr at 37°C. Thick white or yellowish colonies were selected, which was then enriched on tryptic soy agar (TSA, Difco Lab.). Finally, it was tested for identification through coagulase cohesion test and API Staphy (bioMerieux).

S. aureus/Quantification: A quantity (10 g) of the sample was homogenized with 90 mL of peptone water and serially diluted. One-hundred μL of this was inoculated onto MSA agar and then incubated for 48 hr at 37°C. After incubation, thick white or yellowish colonies were selected and counted. An identification test was done using the same method as in isolation and identification.

S. aureus/Detection of enterotoxin: As for the detection of enterotoxin of *S. aureus*, the reversed passive latex agglutination kit (SET-RPLA; Denka Seiken, Tokyo, Japan) was used. SET-RPLA was performed according to the manufacturer's instructions.

S. aureus/PCR analysis for detection of enterotoxin gene: Multiplex PCR was used for detection of enterotoxin genes (2,3). Isolates were streaked on TSA agar and incubated for 20-24 hr at 37°C. Two or three well-isolated colonies were taken using a sterile disposable loop and suspended in double distilled water. The cells were lysed by boiling for 20 min, transferred to ice immediately, and then centrifuged at 10,000×g for 10 min to remove cell debris. The supernatant was used as a DNA template. The PCR mixture was performed in 25 μL reaction volume, containing 5 μL of template DNA, 0.625 U Taq polymerase (TaKaRa Ex Taq™, KaKaRa bio; Otsu, Shiga, Japan), and 20 pmol each of the forward, and reverse primers. The primer used and size of PCR products shown in Table 1. The PCR reaction was run on a DNA thermal cycler of Gene Amp PCR system 9,700 (Applied Biosystems, San Francisco, CA, USA) using the following program, 1 cycle of 5 min at 94°C, 30 cycles of 30 sec at 94°C, 30 sec at 50°C, and 30 sec at 72°C, and finally 10 min at 72°C.

Isolation and identification of *E. coli* For the isolation of *E. coli*, 225 mL of *E. coli* broth (EC broth, Difco Lab.) was added to the 25 g of the sample and was incubated for 24 hr at 37°C. Enriched sample was inoculated onto eosin methylene blue agar (EMB, Difco Lab.) and incubated for 24 hr at 35°C. Typical colonies were counted and selected for further identification. Selected colonies were cultured on triple sugar iron agar (TSI, Difco Lab.) slanted culture medium for 24 hr at 35°C. The yellow isolates producing gas were finally identified as *E. coli* through the VITEK (bioMerieux).

Isolation and identification of *E.coli* O157:H7 A sample of 25 g was taken and 225 mL of *E. coli* O157 enrichment media was added to modified EC broth (Difco Lab.) containing novobiocin and mixed thoroughly for 2-4 min. After being incubated for 18-24 hr at 37°C, it was inoculated on the selective enrichment media sorbitol mackonkey agar (Difco Lab.), then incubated for 18-24 hr. After selecting the typical colony, it was then tested for identification through kligler iron agar (Difco Lab.), VITEK (bioMerieux) and the cohesive reaction of the serum (4,5).

B. cereus/Isolation, identification, and quantification: A quantity (225 mL) of peptone water was mixed with the 25 g of the sample and enriched for 24 hr at 30°C. The sample was serially diluted and inoculated onto MYP agar that was incubated for 24 hr at 30°C. Following the incubation, pink colonies surrounded by a zone of precipitation were counted and selected for further identification by BBL Crystal GP (Becton Dickinson, Franklin Lakes, NJ, USA). The method for quantification of *B. cereus* was performed according to the special edition of Food Code (4).

B. cereus/PCR analysis for detection of eneterotoxin gene: PCR was performed for detection of *B. cereus* enterotoxin gene using the *B. cereus* enterotoxin detection kit (Kogene Biotech, Seoul, Korea). Two or three well-isolated colonies on TSA were selected and suspended in 0.5 mL of distilled water. After centrifugation (5,000×g for 10 min), the supernatant was used as a DNA template. PCR amplification was performed in 25 μL reaction volume in PCR buffer containing 1 μL of each primer and 1 U *Taq* polymerase. The PCR reaction was run on a DNA thermal cycler of Gene Amp PCR system 9,700 (Applied Biosystems) using the following program, 1 cycle of 4 min at 95°C, 30 cycles of 30 sec at 95°C, 30 sec at 55°C, and 30 sec at 72°C, and finally 7 min at 72°C.

V. parahaemolyticus/Isolation and identification: Isolation and identification test for *V. parahaemolyticus* were carried out as described in the special edition of Food Code (4) and Yoon and Kang (6).

V. parahaemolyticus/Quantification: Sample 25 g was added 3% of saline and homogenized and serially diluted. It was spread onto thiosulfate citrate bile salt sucrose agar and incubated for 24 hr at 37°C. The colonies with green precipitation were counted and further identification was done through oxidase test.

Table 1. Primers used for detection of enterotoxin of *S. aureus*

Primer	Sequence	Product size (bp)
SA-A	5'-ATTAACCGAAGGTTCTGT-3'	270
SA-B	5'-ATAGTGACGAGTTAGGTA-3'	165
SA-C	5'-AAGTACATTTTGTAAGTTCC-3'	69
SA-D	5'-TTC GGG AAA ATC ACC CTT AA-3'	306
SA-E	5'-GCCAAAGCTGTCTGA G-3'	213

Table 2. Primers used for detection of *V. parahaemolyticus*

Target gene	Primer sequence	Product size (bp)
*tox*R	5'-AGCCGCCTTTCTTCAGACTC-3' 5'-AACGAGTCTTCTGCATGGTG-3'	399
tdh	5'-TGGTTGACATCCTACATGACTGTG-3' 5'-GGGGATCCCTCAGTACAAAGCCTT-3'	400
trh	5'-GGCTCAAAATGGTTAAGCG-3' 5'-CATTTCCGCTCTCATATGC-3'	250

Table 3. Primers used for detection of *Yesinia* species and pathogenic *Y. enterocolitica* in multiplex PCR

Target gene	Primer sequence	Product size (bp)
ail	5'-TGGTTATGCGCAAAGCCATTGT-3' 5'-TGGAAAGTGGGTTGAATTGCA-3'	356
yst	5'-GTCTTCATTTGGAGGATTCGGC-3' 5'-AATCACTACTGACCTTCGGTGG-3'	134
16S rDNA	5'-GCGGCAGCGGGAAGTAGTTTA-3' 5'-TACAGCGTGGACTACCAGGGT-3'	749

***V. parahaemolyticus*/Identification of toxR, tdh, and trh genes by PCR**: PCR assays for detection of the *tox*R, *tdh* and *trh* genes in *V. parahaemolyticus* were performed. Primers used and size of PCR product were shown in Table 2. PCR conditions were adapted as described by Shirai *et al.* (7).

***C. perfringens*/Isolation, identification, and quantification**: Isolation, identification, and quantification analysis of *C. perfringens* were performed as described in the special edition of Food Code (4). For the final identification of *C. perfringens*, biochemical tests such as Gram-staining, ability of lecitinase production, lactose fermentation, iron-milk test, nitrate reduction test, motility test, verification of α-, β-hemolysis, liquefaction of gelatin, and Rapid ID 32A (bioMerieux) were performed.

***C. perfringens*/PCR assays for detection of α-toxin and enterotoxin genes**: The PCR method for identification of α-toxin and enterotoxin was performed. The primers for detection of α-toxin gene were α1: 5'-TGCTAATGTTACT GCCGTTGAT AG-3' α2: 5'-ATAATCCCAATCATCCCA ACTATG-3'. The primers for detection of enterotoxin gene were CPE 1: 5'-ATCCAATGGTGTTCGAAAAT-3', CPE 2: 5'-ATTTCCTAAGCTATCTGCAG-3'. The PCR product of 247 bp was identified as α-toxin gene and that of 435 bp was identified as an enterotoxin gene.

***Y. enterocolitica*/Isolation and identification**: A quantity (225 mL) of the culture medium, peptone sorbitol bile broth (PSBB, Difco Lab) was added to the 25 g of the sample and incubated for 10 days at 10°C. After 100 μL of the enrichment cultured fluid was added to the 1 mL of saline solution that contained 0.5% of KOH and mixed, it was incubated for 24 hr at 30°C on the cefsulodin irgasan novobiosin agar (CIN, Difco Lab.) after which a deep reddish colony was selected, and inoculated on the side surface and on the top layer of the TSI slanted culture medium, and incubated for 24 hr at 35°C. The isolate that had a yellowish top layer and the side surface which did not have the occurrence of gas and hydrogen sulfide was selected and tested for verification through API 20E (bioMerieux) (4).

***Y. enterocolitica*/Detection of pathogenicity by PCR**: The *ail* (attachment invasion locus) and *yst* (heat stable enterotoxin) gene in chromosome were selected for verifying pathogenic *Y. enterocolitica.* Multiplex PCR with subgenus-specific primer pair was evaluated applicability of differentiating *Yesinia* species from other genus. Primers used and the size of PCR products were shown in Table 3.

***L. monocytogenes*/Isolation and identification**: Isolation and identification of *L. monocytogenes* were performed as described in the special edition of Food Code (4).

***L. monocytogenes*/Quantification**: A quantity (25 g) of the sample was homogenized with 90 mL of saline (0.85%) and serially diluted. One-hundred μL of this was inoculated onto Oxford agar (Difco Lab.) and then incubated for 24 hr at 30°C. After incubation, grey colonies surrounded by a black zone were selected and counted. An identification test was done using the same method as in the special edition of Food codes (4).

***L. monocytogenes*/PCR assay for identification**: Multiplex PCR which was adapted from the method by Yang and Park (8) was used for detection of the enterotoxin gene.

Isolation and identification of *C. jejuni* Isolation and identification of *C. jejuni* were performed as described in special edition of Food Code (4). Identification test was performed through catalase and oxidase test, positive for hippurate test and API Campy (bioMerieux).

Statistical analysis The Dunett's test was used for statistical analysis of the contamination data of foodborne pathogens, using the SigmaStat 2.03 program (Systat Software Inc., San Jose, CA, USA).

Results and Discussion

Detection of foodborne microorganisms in foods A total of 1,240 food samples, including 280 *sashimi* (raw fish fillet), 244 processed frozen products, 258 *kimbab* (cooked rice wrapped with sea weed), 337 soybean pastes, and 121 samples of regionally specialized foods, were examined for 9 major foodborne pathogens. *B. cereus* was the most prominent with 10.55% detected, next were *S. aureus* with 2.7% detected, *V. parahaemolyticus* 2.0%, *C. perfringens* 0.8%, *Y. enterocolitica* 0.2%, and *L. monocytogenes* 0.1%

Table 4. Prevalence of foodborne pathogens in various foods

Bacteria	Number of contaminated samples/ Number of food samples	Proportions (%) of contaminated foods
B. cereus	132/1,240	10.6
S. aureus	34/1,240	2.7
V. parahaemolyticus	25/1,240	2.0
C. perfringens	10/1,240	0.8
Y. enterocolitica	3/1,240	0.2
L. monocytogenes	1/1,240	0.1
Total	205/1,240	16.4

were detected in this order. *Salmonella* spp., *C. jejuni,* and *E. coli* O157:H7 were not found in any of the foods (Table 4). Another prevalence study of foodborne pathogens was conducted in Turkish Van otlu cheese by Tekinşen and Özdemir (9). The foodborne pathogens examined included *S. aureus*, *Salmonella* spp., *E. coli*, *E. coli* O157:H7. Among them, *S. aureus* was found in all samples with high numbers and *E. coli* was found in 62% of the samples whereas only 3 of the 50 samples were contaminated with *Salmonella* spp. and none contained *E. coli* O157:H7.

Detection rate of foodborne microorganisms by regional groups The detection rate of foodborne pathogens was analyzed according to the regions in Korea, and the results are shown in Table 5. The positive detection rate of foodborne pathogens showed slight differences according to the regions in Korea but there were no significant differences between regions ($p<0.05$). Generally, *B. cereus* and *S. aureus* were detected more frequently than the other foodborne pathogens and *E. coli* (Table 5).

Detection rate of foodborne microorganisms by each sample The results of the comparison of detection rates for total foodborne pathogens in each food were demonstrated in Table 6. The results showed that 28.8% of soybean paste, 13.2% *kimbab* & lunch boxes, 21.1% *sashimi*, 11.5% frozen foods were contaminated by foodborne pathogens. Most of foods including soybean pastes were frequently contaminated with *Bacillus* species (10) because of its ubiquitous characteristic (11). In the case of *sashimi*, it was mainly contaminated with *V. parahaemolyticus* but also shown to be contaminated with *B. cereus* and *S. aureus* (Table 6). According to Simon and Sanjeev (12), 21 fishery products among 168 samples collected from India was contaminated with *S. aureus*. Therefore, *B. cereus* and *S. aureus* should also be considered as major contaminants of *sashimi* and fishery products including frozen prawns and fish cutlets.

Analysis of *B. cereus* contamination levels in foods Contamination level of *B. cereus* in soybean pastes was analyzed by the type of soybean pastes and the regional groups, and the results are shown in Table 7. Most soybean products were shown to be contaminated with *B. cereus* regardless of the product type. Among the types of soybean products, mixed soybean pastes collected from Jeju were significantly more contaminated with *B. cereus* when compared to mixed soybean pastes collected from other regions in Korea ($p<0.05$). Contamination levels of *B. cereus* in seafood products were also analyzed by regional groups, and the results are shown in Table 8. The contamination levels of *B. cereus* in seafood from Gyeonggi and Busan were 677 and 7 CFU/g, respectively. According to Yanga *et al.* (13), *B. cereus* is occasionally found in Chinese fermented rice, and Roya *et al.* (14) reported that each of 48 strains of *B. cereus* isolated from

Table 5. Prevalence of foodborne pathogens by regional groups[1)]

Region	No. S	*Sal*	*S.a*	*V.p*	*L.m*	*E.c*	*B.c*	*Y.e*	*C.j*	*C.p*	*EO*
Seoul	232	-	6	-	-	-	22	-	-	1	-
Gyeonggi	112	-	10	-	-	2	36	2	-	6	-
Gwangju	202	-	4	-	-	2	3	-	-	-	-
Daejeon	212	-	1	-	-	3	2	-	-	-	-
Daegu	213	-	5	-	-	4	20	-	-	-	-
Busan	207	-	2	25	1	6	43	1	-	1	-
Jeju	62	-	6	-	-	2	6	-	-	2	-
Total	1,240	0	34	25	1	19	132	3	0	10	0

[1)]No. S, number of samples; *Sal, Salmonella*; *S.a, S. aureus*; *V.p, V. parahaemolyticus*; *L.m, L. monocytogenes*; *E.c, E. coli*; *B.c, B. cereus*; *Y.e, Y. enterocolitica*; *C.j , C. jejuni*; *C.p, C. perfringens*; *EO, E. coli* O157:H7.

Table 6. Prevalence of foodborne pathogens in various foods[1)]

Food	No. S	*Sal*	*S.a*	*V.p*	*L.m*	*E.c*	*B.c*	*Y.e*	*C.j*	*Cl.p*	*EO*
Soybean paste	337	-	1	-	-	3	84	-	-	9	-
Kimbab & lunch box	258	-	20	-	-	-	12	2	-	-	-
Sashimi	280	-	11	25	-	7	14	1	-	1	-
Frozen product	244	-	2	-	1	8	17	-	-	-	-
Total	1,119	0	34	25	1	18	127	3	0	10	0

[1)]No. S, number of samples; *Sal, Salmonella*; *S.a, S. aureus*; *V.p, V. parahaemolyticus*; *L.m, L. monocytogenes*; *E.c, E. coli*; *B.c, B. cereus*; *Y.e, Y. enterocolitica*; *C.j , C. jejuni*; *C.p, C. perfringens*; *EO, E. coli* O157:H7.

Table 7. Detection levels of *B. cereus* in soybean pastes by type of soybean pastes and regional groups

Type of soybean products		Seoul	Gyeonggi	Daejeon	Daegu	Gwangju	Busan	Jeju	Total
Soybean paste	No. S[1)]	24	13	23	26	10	16	5	124
	Average (CFU/g)	1,206	0	0	867	0	2,078	0	721
Red pepper paste	No. S	27	15	0	11	12	8	5	78
	Average (CFU/g)	1,250	220	0	0	238	3,425	4,333	1,141
Fermented soybean paste	No. S	10	0	22	2	0	2	1	37
	Average (CFU/g)	0	0	455	0	0	0	17,000	730
Mixed soybean paste	No. S	32	0	5	12	20	15	5	88
	Average (CFU/g)	2,422	0	0	8	240	2,092	11,550*[2)]	4,719
Chinese soybean paste	No. S	7	0	0	1	0	0	2	10
	Average (CFU/g)	300	0	0	0	0	0	1,625	535

[1)]Number of samples.
[2)]Indicates counts were significantly different at the 5% level.

Table 8. Detection levels of *B. cereus* in seafood products by regional groups

Region	Seoul	Gyeonggi	Daejeon	Daegu	Gwangju	Busan	Jeju
Number of sample	36	35	40	39	40	80	6
B. cereus (CFU/g)	0	677	0	0	0	7	0

Table 9. Detection levels of *S. aureus* in *kimbab* by regional groups

Region	Seoul	Gyeonggi	Daejeon	Daegu	Gwangju	Busan	Jeju
Number of sample	36	26	34	41	37	24	17
S. aureus (CFU/g)	11	167	0	0	187	4	106

6 different kinds of legume-based Indian fermented foods showed resistance against at least nine different antibiotics.

B. cereus is one of the soil-borne microorganisms that is widely distributed in natural environment such as earth, water and dust that has high contingence of infecting food products through original food ingredients. Generally, *B. cereus* forms heat-stable spores that can survive heating at 135°C up to 4 hr (15), therefore in food products or their ingredients that are already infected by this pathogen, the spores will not be destroyed by general heating procedures. This enables it to survive and germinate, so it is inevitable that with current manufacturing processes a certain level of *B. cereus* will be detected in food products such as soybean paste. Foodborne outbreaks associated with *B. cereus* are possible only with over 10^6-10^7 CFU/g of cells (16), however, in this study, the average detection amount in foods was 10^3 CFU/g. This amount is not usually considered to be hazardous to humans.

Analysis of *S. aureus* contamination level in *kimbab*

The contamination level of *S. aureus* in *kimbab* was also analyzed by regional group (Table 9) and the types of shops selling *kimbab* (Table 10). There was no significant difference between regions ($p<0.05$) (Table 9) and *kimbab* samples collected from retail-sale stores were more contaminated with *S. aureus* than those collected from other types of stores selling *kimbab* such as shops in department stores and food halls (Table 10). *Kimbab* is one of the popular ready-to-eat products in Korea and can be contaminated with *S. aureus* because it is usually prepared by hand and stored at room temperature (17,18).

Table 10. Detection levels of *S. aureus* in *kimbab* by type of stores

Type of shop	Department store	Food hall	Retail-sale store
Number of sample	119	44	50
S. aureus (CFU/g)	30	55	122

S. aureus is widespread in nature and one of the representative foodborne pathogens that contaminates through the wounds of human and animals (3,19). From the results of this study, it was shown that the contamination rate of this pathogen is the highest in *kimbab* and other take-out foods that are typically handled frequently. In order to prevent food borne diseases through this pathogen, anyone with pyorrhea should not participate in food processing and preparation and those who prepare the food should handle it after washing hands with disinfectant soap. Also, the usage of disposable gloves should always be practiced so that bare hands do not come into contact with the food. Furthermore, observing general food safety guidelines will help the prevention of contamination.

Characteristics of bacterial isolates from foods *S. aureus* isolates (34 strains) were analyzed for detection of enterotoxin by SET-RPLA and PCR assay for detection of enterotoxin genes. Among the analyzed strains, 7 isolates were shown to produce enterotoxin and all of them

harbored the type A-enterotoxin gene. *B. cereus* isolates (132 strains) were also tested for detection of enterotoxin genes and 81.8% of tested strains (108 strains) were shown to have these genes. These results showed that most strains of *B. cereus* are toxigenic and therefore it should be more strictly controlled in foods. PCR assays targeting the specific genes of *tox*R, *tdh,* and *trh* in *V. parahaemolyticus* were evaluated as a detection method of *V. parahaemolyticus*. These target genes are specific genes related to the pathogenicity of *V. parahaemolyticus*. Therefore, PCR assay targeting on the specific genes of *tox*R, *tdh,* and *thr* could be used for rapid detection of *V. parahaemolyticus*. The results of the PCR assay also showed that none of *V. parahaemolyticus* isolates was toxigenic. The results of PCR assay for detection of α-toxin and enterotoxin genes in *C. perfringens* showed that all isolates of *C. perfringens* (10 strains) harbored α-toxin but did not harbor enterotoxin which is main cause of food poisoning by *C. perfringens*. In the case of *Y. enterocolitica*, multiplex PCR with primers targeting on 16S rDNA showed that it is a good method for differentiating *Y. enterocolitica* from other species because all isolates of *Y. enterocolitica* had amplified products of PCR. Among 3 tested isolates, 2 isolates were shown to harbor pathogenic *yst* gene but none of them had the *ail* gene. Therefore, none of isolates were proved to be pathogenic strains because both *ail* and *yst* genes in *Y. enterocolitica* are needed to cause illness. The isolates that harbor both *hly* and *inl* genes were identified as *L. monocytogenes*.

In conclusion, our studies have shown that the detection level of most food-borne bacteria is much lower than the level needed to cause food-poisoning. Therefore some revisions of the standards for microorgamisms may need to be made for some food items based on risk assessment, particularly for acceptable levels of *B. cereus* in soybean paste.

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