Korean J. Food Sci. Ani. Resour.
Vol. 31, No. 1, pp. 40~46(2011)

ARTICLE

Growth Profile and Toxigenicity of *Bacillus cereus* in Ready-to-eat Food Products of Animal Origin

Mi-Hwa Oh[1], Jun-Sang Ham[1], Kuk-Hwan Seol[1], Aera Jang[1], Seung-Gyu Lee[1], Jong-Moon Lee[1], Beom-Young Park[1], Eun-Sil Kang[2], Ki-Sung Kwon, and In-Gyun Hwang*

National Institute of Food and Drug Safety Evaluation, Korea Food and Drug Administration, Cheongwon 363-951, Korea

[1]National Institute of Animal Science, Rural Development Administration, Suwon 441-744, Korea

[2]Ildongfoodis R&D Center, Chuncheon 200-881, Korea

Abstract

The growth profile of *Bacillus cereus* in ready-to-eat (RTE) food products of animal origin was examined under different temperature and incubation conditions. In sandwiches and *Kimbab*, *B. cereus* did not grow or exhibited only minimal growth at 4 and 10°C, but it grew rapidly at ambient temperature. In sandwiches, *B. cereus* did not grow efficiently at 25°C, however, in ham, the main ingredient of sandwiches, *B. cereus* growth was observed at the same temperature, with bacterial levels reaching 7.94 Log CFU/g after incubation for 24 h at 25°C. Toxigenicity of *B. cereus* was observed only at temperatures above 25°C. In *Kimbab*, *B. cereus* produced toxin after 9 h at 30°C and after 12 h at 25°C. Ingredients of sandwiches and *Kimbab* were collected from 3 different Korean food-processing companies to investigate the source of contamination by *B. cereus*. Among the 13 tested food items, 6 items including ham were found to be contaminated with *B. cereus*. Of these ingredients, *B. cereus* isolates from 3 items produced enterotoxins. None of these isolates harbored the emetic toxin-producing gene. The findings of the present study can be used for risk assessments of food products, including ham and cheese, contaminated with *B. cereus*.

Key words: *Bacillus cereus*, growth profile, toxigenicity, enterotoxin, emetic toxin

Introduction

Although substantial progress has been achieved in the food industry and in food safety management, the incidence of food poisoning remains a major problem in this industry in terms of social and economic losses. Animal-derived food products have been the primary cause of food poisoning outbreaks in Korea for the last 11 years (Bahk, 2009), and recently, in line with the increased consumption of ready-to-eat (RTE) foods, the incidence of food poisoning related to these foods has increased (Bahk *et al*., 2007). *Kimbab* and sandwiches are the most common RTE foods in Korea, and ham and cheese are their major ingredients.

Bacillus cereus is a soil-borne microorganism that is widely distributed in the natural environment (soil, water, and dust); and therefore, is also found in foodstuffs of vegetable and animal origin. Many investigators have reported that *B. cereus* is a contaminant of not only raw meat (Johnson, 1984; Nel *et al*., 2004), but also processed meat products (Sooltan *et al*., 1987; Nortjé *et al*., 1999). Psychrotrophic strains of *B. cereus* can also contaminate refrigerated foods, such as RTE foods. Some strains of *B. cereus* can grow at temperatures below 10°C. These strains have mainly been isolated from dairy products (Andersen Borge *et al*., 2001; van Netten *et al*., 1990).

B. cereus is difficult to eliminate from foodstuffs and food-processing systems because it forms spores that are ubiquitous and highly resistant to adverse conditions such as heat, dehydration, and other physical stresses (Andersson, 1995; Doan and Davidson, 1999; Larsen and Jørgensen, 1999). *B. cereus* is associated with 2 distinct types of gastrointestinal disorders: diarrheal and emetic syndromes (Beattie and Williams, 2000; Schoeni and Wong, 2005). The first and better understood is the diarrheal syndrome caused by *B. cereus* enterotoxins; this syndrome is associated with diarrhea and abdominal pain

*Corresponding author: In-Gyun Hwang, Food Microbiology Division, National Institute of Food and Drug Safety Evaluation, Korea Food and Drug Administration, Cheongwon 363-951, Korea. Tel: 82-43-719-4301, Fax: 82-43-719-4315, E-mail: inghwang@kfda.go.kr

occurring 8-16 h after the ingestion of contaminated foods. The emetic syndrome is caused by the emetic toxin of *B. cereus* and is characterized by nausea and vomiting occurring 1-5 h after the ingestion of contaminated foods.

In this study, we examined the growth patterns and toxigenicity of *B. cereus* in foodstuffs of animal origin, such as ham and cheese, in RTE sandwiches and *Kimbab*, and analyzed these ingredients to identify the major sources of *B. cereus* contamination and thereby improve the management of *B. cereus* contamination of RTE foods.

Materials and Methods

B. cereus strains

To choose the strain of *B. cereus* used for generating growth pattern and analyzing toxin production according to differences in temperature and growth matrix, the toxin production of five *B. cereus* reference strains (ATCC 11778, KCTC 1092, KCTC 1094, KCTC 1013, and KFRI 181) was tested. The diarrhoeal type *B. cereus* enterotoxin test kit (RPLA kit; Oxoid, England) was used to examine toxin production according to the manufacturer's instructions as described in Fig. 1. Among the five strains tested, *B. cereus* KTCT 1013 was selected for use in the remainder of the experiments.

Food materials for the growth profile of *B. cereus*

After testing a variety brands of sandwich and *Kimbab* samples, those found to be uncontaminated with viable microorganisms were chosen as matrices for generating growth patterns for *B. cereus*. Chosen sandwiches and *Kimbab* not inoculated with bacteria were served as samples for each experiment.

Growth profile of *B. cereus*

B. cereus KTCT 1013 was grown in tryptone soya broth (Oxoid) for 20 h prior to inoculation. *B. cereus* (1-2$\times10^3$ CFU/g) was used to inoculate a slice of *Kimbab* (20 g) and it was left for 20 min to be absorbed. For the sandwiches and sandwich ingredients (ham and cheese), 1-2$\times10^3$ CFU/g of *B. cereus* was inoculated onto 20 g of sandwich slice or ingredients, which were left for 20 min to be absorbed. The inoculated samples were homogenized with 180 mL of sterilized saline (0.85%) by stomaching (Stomacher 400, UK) for 2 min. Then, the samples were serially diluted and inoculated onto mannitol-egg yolk-polymyxin agar (Oxoid), and incubated for 24-48 h at 35°C. Following incubation, pink colonies surrounded by a zone of precipitation were counted and selected for identification by BBL-crystal Gram-positive (GP; Becton Dickinson, USA) analysis and 16S rDNA sequencing.

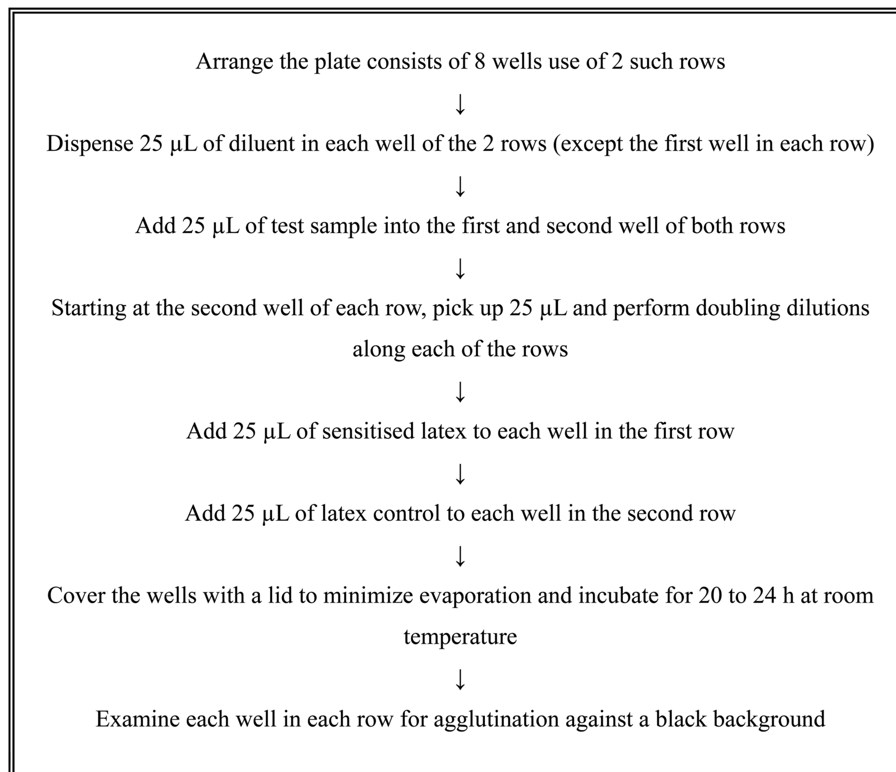

Fig. 1. RPLA procedure for detection of diarrhoeal toxin.

The growth and toxin production of *B. cereus* in *Kimbab* and BHI broth (Oxoid) was analyzed at 4, 10, 25, and 30°C. Incubation times were 0, 3, 6, 9, 12, 18, and 24 h. For sandwiches and sandwich ingredients (ham and cheese), the growth was analyzed at 10 and 25°C for 0, 3, 6, 9, 12, 18, and 24 h. At each time point, the number of viable cells was counted and toxin production was analyzed.

Examination of *B. cereus* contamination in RTE ingredients

To examine contamination sources of ingredients, three different food processing companies in Korea (A, B, and C) were selected and collected ingredients used for *Kimbab* (rice rolled in dried laver) and sandwiches. The 13 ingredients (n=37) of *Kimbab* and sandwiches are including ham, cheese (for both *Kimbab* and sandwiches), dried laver, steamed rice, carrot, pickled radish, cucumber, tuna, bread, lettuce, dried gourd, salad and tomato. The level of *B. cereus* contamination of each ingredient was determined as follow; 20 g of samples were homogenated with 180 mL of sterilized sarline (0.85%) for 2 min, then the samples were serially diluted and inoculated onto mannitol-egg yolk-polymyxin agar (Oxoid), and incubated for 24-48 h at 35°C. Following incubation, pink colonies surrounded by a zone of precipitation were counted and selected for identification by BBL-crystal Gram-positive (GP; Becton Dickinson, USA) analysis and 16S rDNA sequencing.

Confirmation of *B. cereus* isolated from food ingredients

BBL-Crystal GP (Becton Dickinson, USA) confirmation of *B. cereus* isolated from food ingredients was performed according to the manufacturer's instructions. For sequencing, DNA of *Bacillus* strains was isolated using the DNeasy® Blood and Tissue kit (Qiagen, USA) and PCR amplification was carried out as described by Nübel *et al*. (1996) and Garbeva *et al*. (2001) using the universal primers 968F (5'-AACGCGAAGAACCTTAC-3'; Eubacteria V6; Heuer *et al*., 1999) and 1401R (5'-CGGTGTGTACAAGACCC-3'; Eubacteria V9; Nübel *et al*., 1996). PCR amplification was performed in a reaction volume of 50 μL containing: 1×PCR buffer, 0.2 μM of each primer, 200 μM of each dNTP, 1.5 mM $MgCl_2$, 1.25 U of *Taq* DNA polymerase and 10 ng of extracted DNA. The PCR was performed using a DNA thermal cycler (Gene Amp PCR system 9700) (Applied Biosystems, USA). The temperature cycles comprised initial denaturation at 94°C for 2 min, 10 cycles at 94°C for 30 s, 50°C for 30 s, and 72°C for 45 s, followed by 20 times the same cycle with each successive cycle at 5 s longer elongation time. The final elongation was conducted for 10 min at 72°C. PCR amplicons were visualised by gel electrophoresis and PCR products of interest were used for sequence analysis. Sequencing of *B. cereus* isolates was carried out with the ABI PRISM® BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) and submitted to Bioneer, Inc. (Korea) for analysis.

Toxigenicity of *B. cereus* isolates

The samples were prepared by inoculating a loopful of biomass from a 24 h culture on tryptone soya agar (Oxoid) into 10 mL of brain heart infusion (BHI, Oxoid) broth, incubated for 16-18 h at 35-37°C. Analysis of enterotoxin production was conducted using the RPLA kit according to the manufacturer's instructions as described in Fig. 1. PCR was performed to detect the emetic toxin gene. The primer set and the PCR conditions for detection of the emetic toxin gene were as described by Toh *et al*. (2004). The PCR primers used were BE F (5'-ACTTAGATGATGCAAGACTG-3') and BE R (5'-TTCATAGGATTGACGAATTTT-3'). PCR amplification was performed in a reaction volume of 30 μL containing 3 μL $MgCl_2$, 3 μL 10 × buffer, 2 μL dNTPs, 0.1 μL *Taq* polymerase, 1 μL each of the forward and reverse primer in 19 μL Milli-Q water, and 1 μL of DNA was included in the reaction volume. The amplification was performed using 30 cycles, each consisting of 10 s at 94°C, 20 s at 50°C, and 80 s at 72°C, with a final extension step at 72°C for 7 min. PCR products were analysed by gel electrophoresis in 1% (w/v) agarose gels in TBE buffer and visualized by staining with ethidium bromide.

Statistical analysis

All experiments for growth profile of *B. cereus* were replicated three times. Statistical analysis (ANOVA) of data was performed using the SigmaStat 2.03 program (Sigma-Aldrich, USA). Duncan's multiple range test was used to analyze the statistical significance of the means.

Results

Analysis of *B. cereus* growth profile and toxigenicity in RTE foods

Among the strains tested, such as *B. cereus* ATCC 11778, KCTC 1092, KCTC 1094, KCTC 1013 and KFRI 181, *B. cereus* KTCT 1013 showed the highest toxin pro-

duction (data not shown) and was selected for inoculation.

As illustrated in Fig. 2, *B. cereus* grew only slightly in *Kimbab* at 4 and 10°C. The bacteria was inoculated at 3.11 Log CFU/g and after 24 h at 4 or 10°C, the bacterial counts were 3.40 and 3.66 Log CFU/g, respectively. There were significant differences in the bacterial counts between 0 and 24 h at 4 and 10°C (p<0.05). When grown at higher temperatures, number of *B. cereus* increased dramatically to 5.80 Log CFU/g at 25°C and to 6.91 Log CFU/g at 30°C after 24 h (Fig. 2).

No toxin production was detectable in the bacteria grown at 4 and 10°C; the samples were negative for toxin production at all stages tested until 24 h. However, at 25 and 30°C, all samples started to produce toxin after 12 and 9 h, respectively (Fig. 2). In comparison, when *B. cereus* was grown in BHI broth, toxin production was similar to that in *Kimbab* at 4, 10, and 25°C. However, in BHI at 30°C, *B. cereus* began producing toxin after 12 h, which was later than in *Kimbab* (data not shown).

B. cereus did not grow rapidly in sandwiches at 25°C. As illustrated in Fig. 3, there was no significant growth of *B. cereus* in the sandwich during 24 h of storage at 10°C (p>0.05), whereas there was slight growth in *Kimbab* under these same conditions. Even though the growth rate of *B. cereus* in sandwiches was significantly different at hour-0 versus hour-12 at 25°C (p<0.05), the number of *B. cereus* increased only from 3.80 Log CFU/g (inoculation concentration) to 3.98 Log CFU/g.

Therefore, to determine the ingredients that posed a greater risk for bacterial contamination, the growth patterns of *B. cereus* in the most frequently used ingredients for sandwiches and *Kimbab* such as ham and cheese were examined (Fig. 4). In the ham, there was no change in

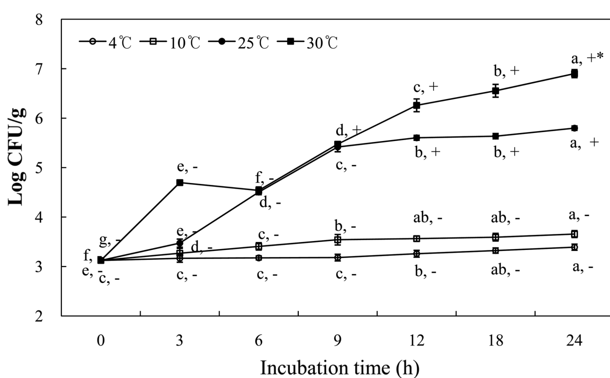

Fig. 2. Growth patterns of *B. cereus* at various temperatures in *Kimbab*. *[a-g]In same sample with different superscripts are significantly different (p<0.05). + or - means the toxin-production of *B. cereus*.

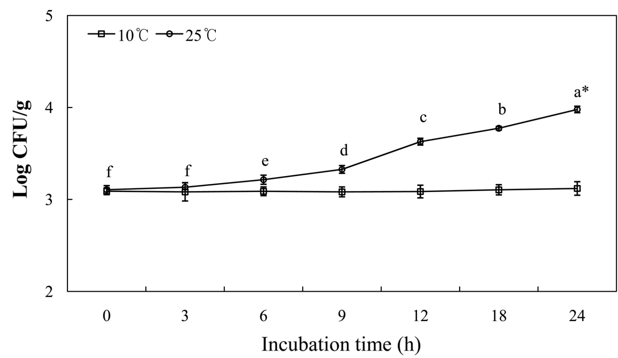

Fig. 3. Growth patterns of *B. cereus* at 10 and 25°C in sandwiches. *[a-f]In same sample with different superscripts are significantly different (p<0.05).

number of *B. cereus* after the inoculation of 3.32 Log CFU/g at 10°C. However, at 25°C, the growth rate of *B. cereus* increased rapidly between 6 and 9 h and the number of *B. cereus* increased from 3.72 Log CFU/g to 4.08 Log CFU/g, ultimately reaching 7.94 Log CFU/g at 24 h representing the higher level of growth of *B. cereus* than cheese (Fig. 4). In contrast, *B. cereus* did not grow in the cheese regardless of incubation temperature (Fig. 4).

In whole sandwich samples, *B. cereus* grew only to the level of 3 Log CFU/g and did not produce enterotoxin in any stage, thus it was not possible to evaluate toxin pro-

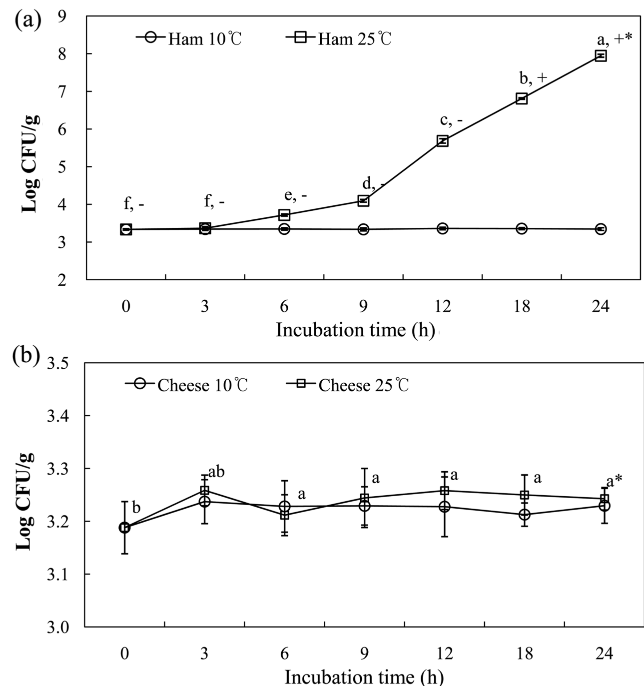

Fig. 4. Growth patterns of *B. cereus* at 10 and 25°C in ham (a) and cheese (b). *[a-f]In same sample with different superscripts are significantly different (p<0.05). + or - means the toxin-production of *B. cereus*.

duction according to the growth of *B. cereus* in sandwiches (Fig. 3). Therefore, we evaluated toxin production in ham, which supported the greatest *B. cereus* growth of all sandwich ingredients (Fig. 4a). There was no enterotoxin production detected when the samples were grown at 4 or 10°C for 24 h. However, at 25 and 30°C, all samples started to produce toxin by 18 h.

B. cereus contamination in food ingredients of RTE foods

To determine the sources of contamination of ingredients, we visited three food companies and collected 13 ingredients (n=37) of *Kimbab* and sandwiches and evaluated *B. cereus* contamination in those samples. Isolates from samples were confirmed as *B. cereus*-positive after BBL-crystal GP analysis and sequencing showed 99% similarity to *B cereus* (data not shown). *B. cereus* was detected in ham and bread used for sandwiches and in cucumber, carrot, and laver for *Kimbab* (Table 1).

Toxigenicity of *B. cereus* isolated from food ingredients of RTE foods

The results of enterotoxin production evaluation and screening for the presence of the emetic toxin-producing gene in the *Bacillus* strains isolated from food ingredients are shown in Table 2. *B. cereus* isolates from ham didn't produce enterotoxin. However, all *B. cereus* isolates from cucumber collected from two different companies and one isolates from bread produced enterotoxin. There is no commercially available kit that screens for the presence of emetic toxin. Therefore, PCR amplification was used to detect the emetic toxin-producing gene; however, none of isolates was found to harbor this gene (Fig. 5).

Table 1. Screening of *B. cereus* existence from *Kimbab* and sandwich ingredients

Food ingredient		Contamination level (Log CFU/g)		
		Company A	Company B	Company C
Kimbab	Steamed rice	ND	ND	2.78
	Ham	ND	-	ND[1]
	Cheese	-	-	ND
	Dried laver	ND	3.48	$< 10^2$
	Pickled radish	ND	ND	ND
	Cucumber	< 2	ND	$< 10^2$
	Carrot	ND	2.30	-
	Dried gourd	ND	ND	-
Sandwich	Bread	< 2	ND	< 2
	Ham	ND	< 2	ND
	Cheese	ND	ND	-
	Salad	ND	ND	ND
	Lettuce	ND	ND	ND
	Tomato	ND	-	ND
	Tuna	-	ND	ND

[1]ND, not detected; -, not tested

Table 2. Toxigenicity of *B. cereus* isolated from food ingredients

Food ingredient		Company	Enterotoxin production[1]	Emetic toxin gene
Kimbab	Rice	C	-	-
	Dried laver	B	+	-
		C	-	-
	Cucumber	A	+	-
		C	+	-
	Carrot	B	-	-
Sandwich	Bread	A	-	-
		C	+	-
	Ham	B	-	-

[1]+, Positive results; -, negative results

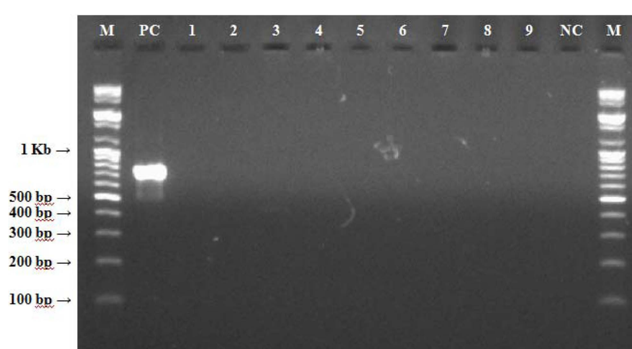

Fig. 5. Electrophoretic analysis of *B. cereus* emetic toxin gene. M, DNA markers; PC, positive control (*B. cereus* F4810/72); 1-6, *B. cereus* isolated from food ingredients of *Kimbab* (1, rice from company C; 2, dried laver from company B; 3, dried laver from company C; 4, cucumber from company A; 5, cucumber from company C; 6, carrot from company B); 7-9, *B. cereus* isolated from food ingredients of sandwich (7, bread from company A; 8, bread from company C; 9, ham from company B).

Discussion

We examined the growth profile, contamination sources, and toxigenicity of *B. cereus* from ham and cheese in RTE sandwiches and *Kimbab* in order to identify control point to minimize *B. cereus* contamination and improve food safety management. Because of the ubiquitous nature of this organism, *B. cereus* can contaminate raw meat exposed to sources such as soil, hide, equipment, and personnel (Nel *et al.*, 2004). Further, meat products containing additives such as spices, seasonings, proteins,

starch, and colorants are more likely to be exposed to *B. cereus* because these additives have also been shown to be contaminated with *B. cereus* (Shinagawa *et al*., 1988).

In this study, we identified 2 main control points for preventing food poisoning by *B. cereus* contamination of RTE sandwiches and *Kimbab*. The first point of control is the storage and delivery temperature of foods, which must be rigorously maintained. We found that if the storage temperature is maintained below 10°C, *B. cereus* does not grow efficiently, whereas it grows rapidly at temperatures above 25°C (Fig. 2 and Fig. 4a). Only 1 company among the 3 that we visited used a cold system for the entire *Kimbab* and sandwich preparation process. The second point of control is the initial pathogen contamination level. Because *B. cereus* can survive at 4°C, the bacteria can begin to multiply if the storage conditions are changed to those more conducive to its growth. In sandwiches, the growth rate of *B. cereus* was very slow at 25°C (Fig. 3), in contrast to its growth rate in *Kimbab* (Fig. 2). However, at 25°C, *B. cereus* grows efficiently in ham, one of the main ingredients of sandwiches (Fig. 4a). Therefore, it is important to control the initial contamination levels of the ingredients used to prepare RTE sandwiches and *Kimbab*.

Our data showed that the ingredients of sandwiches and *Kimbab*, including ham, steamed rice, bread, dried laver, cucumbers, and carrots, were contaminated with *B. cereus*. In food poisoning, the infective dose of diarrheal *B. cereus* ranges from 4 to 11 Log CFU/g (or mL; Granum and Lund, 1997), and in emetic food poisoning, the infective dose of *B. cereus* ranges from 3 to 10 Log CFU/g (or mL; Lund, 1990). It is generally thought that any food product with a *B. cereus* concentration exceeding 4 to 5 Log cells or spores/g (or mL) is unsafe for consumption (Beattie and Williams, 2000; Granum, 2002), although foods with *B. cereus* concentrations higher than these values have been consumed without incident (Häggblom *et al*., 2002; Notermans and Batt, 1998). Therefore, even though none of sample was contaminated at a level higher than 4 Log CFU/g, the products cannot be described as safe. If the storage temperature is not properly controlled, *B. cereus* can multiply and produce toxins.

The toxin-producing strains of *B. cereus* must be managed carefully because *B. cereus* can produce emetic toxin and 5 types of diarrheal toxins (enterotoxins; Granum, 2002). There are 2 commercial immunoassay kits available for detecting enterotoxins. The test kit for enterotoxins produced by diarrheal type *B. cereus* (RPLA kit; Oxoid) is specific for the HblC (L_2) component, whereas the *Bacillus* Diarrhoeal Enterotoxin Visual Immunoassay kit (BDE kit; Tecra) detects mainly the NheA (45-kDa) protein (Granum and Lund, 1997). We used the RPLA kit in this study because Hbl was suggested to be a primary virulence factor in *B. cereus* diarrhea (Beecher *et al*., 1995). We found that enterotoxin production by *B. cereus* inoculated into *Kimbab* was faster than that in ham at 25°C, indicating that differences in the matrix can affect toxin production (Fig. 2 and Fig. 4a). However, toxin production may not be correlated to the number of cells. In our study, the *B. cereus* cell count was higher in ham (7 Log CFU/g after incubation for 12 h at 25°C and 30°C) than in *Kimbab* (~6 Log CFU/g at same temperature) (Fig. 2 and Fig. 4a). However, toxin production was faster in *Kimbab* (after 12 h at 25°C) than in ham (after 18 h at 25°C) (Fig. 2 and Fig. 4a). Therefore, we concluded that toxin production is related more to the culture medium and temperature than to the cell number.

The results generated from this systematic comparison of *B. cereus* growth and toxin production in ham and cheese in sandwiches and *Kimbab* according to storage temperature and time. It was found that ham is important ingredient in the respect of food safety because it supports faster growth of *B. cereus* than that in *Kimbab*. The growth profiles of *B. cereus* in ham and chesses, and the evaluation of RTE ingredients as sources of contamination can be used to establish risk assessments for bacilli.

References

1. Andersson, A. (1995) *Bacillus cereus*, strategy for survival - a literature review. SIK report No. 612, Gothenburg, Sweden.
2. Anderson Borge, G. I., Skeie, M., Sørhaug, T., Langsrud, T., and Granum, P. E. (2001) Growth and toxin profiles of *Bacillus cereus* isolated from different food sources. *Int. J. Food Microbiol*. **69**, 237-246.
3. Bahk, G. J. (2009) Determining of Risk Ranking for Processed Foods in Korea. *J. Food Hyg. Safety* **24**, 200-203.
4. Bahk, G. J., Todd, E. C. D., Hong, C. H., Oh, D. H., and Ha, S. D. (2007) Exposure assessment for *Bacillus cereus* in ready-to-eat Kimbab selling at stores. *Food Control* **18**, 682-688
5. Beattie, S. H. and Williams, A. G. (2000) Detection of toxins. In: Encyclopedia of food microbiology. Robinson, R. K., Batt, C. A., Patel, P. D. (eds) Academic Press, A Harcourt Science and Technology Company, London, pp. 141-148.
6. Beecher, D. J., Schoeni, J. L., and Wong, A. C. L. (1995) Enterotoxin activity of hemolysin BL from *Bacillus cereus*. *Infect. Immun.* **63**, 4423-4428.
7. Doan, C. H. and Davidson, P. M. (1999) Growth of *Bacillus cereus* on oil-blanched potato strips for "home-style" French

fries. *J. Food Sci.* **64**, 909-912.

8. Garbeva, P., van Overbeek, L. S., van Vuurde, J. W. L., and van Elsas, J. D. (2001) Analysis of endophytic bacterial communities of potato by plating and denaturing gradient gel electrophoresis (DGGE) of 16S rDNA based PCR fragment. *Microb. Ecol.* **41**, 369-383.
9. Granum, P. E. and Lund, T. (1997) *Bacillus cereus* and its food poisoning toxins. *Microbiol. Lett.* **157**, 223-228.
10. Granum, P. E. (2002) *Bacillus cereus* and food poisoning. In: Applications and systematics of *Bacillus* and relatives. Berkeley R, Heyndrickx M, Logan N., and De Vos P. (eds) Blackwell Science Ltd., UK, pp. 37-46.
11. Häggblom, M. M., Apetroaie, C., Andersson, M. A., and Salkinoja-Salonenm, M. S. (2002) Quantitative analysis of cereulide, the emetic toxin of *Bacillus cereus*, produced under various conditions. *Appl. Environ. Microbiol.* **68**, 2479-2483.
12. Heuer, H., Hartung, K., Wieland, G., Karmer, I., and Smalla, K. (1999) Polynucleotide probes that target a hypervariable region of 16S rRNA genes to identify bacterial isolates corresponding to band of community fingerprints. *Appl. Environ. Microbiol.* **65**, 1045-1049.
13. Johnson, K. M. (1984) *Bacillus cereus* foodborne illness: An update. *J. Food Prot*. **47**, 145-153.
14. Larsen, H. D. and Jørgensen, K. (1999) Growth of *Bacillus cereus* in pasteurized milk products. *Int. J. Food Microbiol.* **46**, 173-176.
15. Lund, B. M. (1990) Food-borne disease due to *Bacillus* and *Clostridium* species. *Lancet* **336**, 982-986.
16. Nel, S., Lues, J. F. R., Buys, E. M., and Venter, P. (2004) Bacterial populations associated with meat from the deboning room of a high throughput red meat abattoir. *Meat Sci*. **66**, 667-674.
17. Nortermans, S. and Batt, C.A. (1998) A risk assessment approach for food-borne *Bacillus cereus* and its toxins. *J. Appl. Microbiol.* **84**, 51s-61s
18. Nortjé, G. L., Vorster, S. M., Greebe, R. P., and Steyn, P. L. (1999) Occurrence of *Bacillus cereus* and *Yersinia enterocolitica* in South African retail meats. *Food Microbiol*. **16**, 213-217.
19. Nübel, U., Engelen, B., Felske, A., Snaidr, J., Wieshuber, A., Amann, R. I., Ludwig, W., and Backhaus, H. (1996) Sequence heterogeneities of genes encoding 16S rRNAs in *Paenibacillus polymyxa* detected by TGGE. *J. Bacteriol.* **178**, 5636-5643.
20. Schoeni, J. L. and Wong, A. C. L. (2005) *Bacillus cereus* food poisoning and its toxins. *J. Food Prot.* **68**, 636-648.
21. Shinagawa, K., Konuma, H., Tokumaru, M., Takemasa, N., Hashigiwa, M., Shigehisa, T., and Lopes, C. A. M. (1988) Enumeration of aerobic spore-formers and *Bacillus cereus* in meat product additives. *J. Food Prot*. **51**, 648-650.
22. Sooltan, J. R. A., Mead, G. C., and Norris, A. P. (1987) Incidence and growth potential of *Bacillus cereus* in poultry meat products. *Food Microbiol*. **4**, 347-351.
23. Toh, M., Moffitt, M. C., Henrichsen, L., Raftery, M., Barrow, K., Cox, J. M., Marquis, C. P., and Neilan, B. A. (2004) Cereulide, the emetic toxin of *Bacillus cereus*, is putatively a product of nonribosomal peptide synthesis. *J. Appl. Microbiol.* **97**, 992-1000.
24. van Netten, P., van de Moosdijk, A., van Hoensel, P., Mossel, D. A. A., and Perales, I. (1990) Psychrotrophic strains of *Bacillus cereus* producing enterotoxin. *J. Appl. Bacteriol*. **69**, 73-79

(Received 2010.7.26/Revised 2010.12.16/Accepted 2011.1.4)