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Prevalence of *Bacillus cereus* Group in Rice and Distribution of Enterotoxin Genes

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Abstract *Bacillus cereus* group comprising *B. cereus, B. thuringiensis*, and *B. mycoides* was differentiated by polymerase chain reaction (PCR) and colony morphology. Prevalence of *B.cereus* group in rice and distribution of enterotoxin genes were determined as possible food poisoning agents. PCR using primers targeted for *gyrB* and *cry* genes could distinguish *B. thuringiensis* from *B. cereus*, and *B. mycoides* was differentiated by rhizoid morphological characteristics on nutrient agar. Among 136 rice and their processed products, prevalence of *B. cereus* group was 40%. *B. cereus* group consisted of 54 *B. cereus*, 11 *B. thuringiensis*, and 1 *B. mycoides*. Major isolates were *B. cereus*, with *B. thuringiensis* detected up to 10% among edible rice tested. Five enterotoxin genes, *hbl, nhe, bceT, entFM*, and *cytK*, were broadly distributed among *B. cereus* group, especially in *B. cereus* and *B. thuringiensis*. Prevalence of *B. cereus* group in rice and enterotoxin distribution suggest *B. thuringiensis* and *B. cereus* are toxigenic strain that should be controlled in rice and its products.

Keywords: *Bacillus cereus* group, *Bacillus cereus*, *Bacillus thuringiensis*, prevalence in rice, enterotoxin distribution, PCR

Introduction

Bacillus cereus group, comprising *B. cereus, B. thuringiensis*, and *B. mycoides* based on classification as food-borne pathogen or spoilage organism, are very closely related bacteria known to cause food poisoning or spoilage, because they can survive heat treatment (1). Food-borne diseases caused by *B. cereus* are classified as emetic and diarrheal syndromes. Emetic syndrome is caused by only one toxin, cereulide, which causes vomiting. Other strains are known to produce extracellular enterotoxins, which cause diarrhea (2). Five different enterotoxins have been described from the *B. cereus* group, two protein complexes, hemolysin BL (HBL) and non-hemolytic enterotoxins (NHE), and three single gene products encoded by *entFM, cytK*, and *bceT* (3-6). The most investigated enterotoxin is HBL, a three-component hemolysin, which consists of two lytic components (L_1 and L_2) and a binding component (B) (7). NHE is another three-component enterotoxin complex that was originally identified as a *B. cereus* strain responsible for food-poisoning outbreak (8). In addition, one-component toxins, such as enterotoxins T (6) and FM (4), as well as cytotoxin K (6) are also thought to be involved in *B. cereus* food poisoning.

B. thuringiensis produces intracellular and crystal proteins during sporulation. These parasporal proteins have insecticidal activity specific to certain groups of insects (9). *B. mycoides*, a common soil microorganism, are mostly non-motile and shows a rhizoid growth, which is characterized by forming colonies with long hair or root-like structures (10). *B. cereus* and *B. thuringiensis* cannot be differentiated on the basis of biochemical characteristics, and the nucleotide sequences of 16S rRNAs of the *B. cereus* group exhibit very high levels of sequence similarity (99%), consistent with the close relationships shown by previous DNA hybridization studies (11). Such similarities between *B. thuringiensis* and *B. cereus* require more selective detection method. *B. mycoides* and *B. thuringiensis* have also shown positive reaction via a commercial reverse passive latex agglutination (RPLA) assay for the production of enterotoxins (12). *B. thuringiensis* has recently been reported to be involved in the outbreaks of gastrointestinal diseases, some of which have been reported to produce enterotoxins by a number of different techniques (13). Furthermore, some *B. thuringiensis* strains have been reported to possess genes known to be involved in *B. cereus* pathogenesis (14). Toxin production of the widely distributed *B. thuringiensis* and the prevalent soil bacterium *B. mycoides* must be critically considered in food microbiology.

Lately, the new regulation for safety of the ready-to-eat foods by the number of *B. cereus* is inforced to control *B. cereus* poisoning by the governmental agency in Korea. However, no clear method has yet been developed to isolate and differentiate strains among *B. cereus* group, especially between *B. cereus* and *B. thuringiensis*. Results of biochemical characteristics obtained using the mannitol-egg yolk-polymixin (MYP) selective medium are too similar to be applied for differentiation (15). Such the method should be also able to detect enterotoxins causing food poisoning among *B. cereus* group. As yet, little information is available on the prevalence of *B. cereus* group needed to control the safety of foods in Korea.

In this study, we applied sets of polymerase chain reaction (PCR) primers to the differentiation of *B. cereus* group and analyzed the morphological characteristics on the media. Distribution of the five enterotoxin genes reported were analyzed for grouping of the isolates from Korean rice, and the prevalence of *B. cereus* group was evaluated.

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Materials and Methods

Strains and rice samples The strains used were *B. cereus* KCCM40935, KCCM11773, and KCTC1094, *B. thuringiensis* KCTC1509, KCTC3452, and KCCM11428, *B. thuringiensis* subsp. *kurstaki* KCCM11429, and *B. mycoides* KCTC3453. Other *B. cereus* group strains were isolated from raw rice and its processed rice using the selective medium, and identified by PCR and biochemical tests. Unhusked rice samples (31) were collected directly from local farms in Kyonggi-Do, Korea, and 26 brown rice, 44 *kimbap*, and 35 Japanese vinegared rice delicacy samples were purchased from markets in Seoul and Kyonggi-do.

Isolation of *B. cereus* group *B. cereus* group was isolated from the food samples using the MYP agar with 5% egg-yolk emulsion (Difco Laboratories, Livonia, MI, USA) and polymixin B 20,000 IU (Sigma, St. Louis, MO, USA). Samples were inoculated onto MYP agar, and plates were incubated at 37°C for 24-48 hr. Typical pink opaque halo colonies were collected from the plates and analyzed for Gram-staining, catalase activity, hemolysis, and morphology.

Polymerase chain reaction (PCR) PCR was operated for the rapid identification of *B. cereus* group, and the primers were synthesized (Genotech and Bioneer, Taejon, Korea) (Table 1) (16). The primers of *gyrB, cry1A, hbl, nheA, bceT, entFM,* and *cytK* came from Manzano *et al.* (17), Bourque *et al.* (18), Mantynen *et al.* (19), Granum *et al.* (20), Agata *et al.* (4), Asano *et al.* (5), and Lund *et al.* (6), respectively. The whole cells of *B. cereus* group were used as the DNA templates for PCR without DNA extraction. They were centrifuged, the supernatant removed, and resuspended in sterilized distilled water of 1/10 volume, and heated for 5 min at 100°C. The cell lysate was used as a template. PCR was performed at the reaction volume of 20 μL with 1 U thermostable DNA polymerase and 10 pmole of each primer in 250 μM dNTPs, 50 mM Tris-HCl (pH 8.3), 40 mM KCl and 1.5 mM $MgCl_2$, and 2 μL cell lysate. The reaction tube was moved to the gene cycler (BioRad, Hercules, CA, USA). The amplification of *gyrB* gene was performed by 30 cycles PCR, each consisting of 1 min at 94°C, 1.5 min at 58°C, and 2.5 min at 72°C, with a final extension step at 72°C for 7 min. The other amplification conditions were followed in above reports for each primer.

***B. mycoides* morphological characterization** *B. mycoides* was inoculated into 10 mL brain heart infusion (BHI), cultured overnight, and identified based on morphological characteristics on nutrient agar (Difco Laboratories).

Enterotoxin production assays Enterotoxin production by *B. cereus* group was determined using enterotoxin reverse passive latex agglutination test kit (Oxoid, Basingstoke, England) and the *Bacillus* diarrheal enterotoxin visualimmunoassay kit (Tecra Diagnostics, Roseville, Australia) according to the directions supplied by the manufacturers.

Table 1. The primers used to detect the enterotoxin genes from *B.cereus* group with PCR in this study

Target gene	Primer	Oligonucleotide sequence (5'-3')[1]	Product size (bp)
gyrB	BC1/BC2r	5'-ATTGGTGACACCGATCAAACA-3'/(F) 5'-TCATACGTATGGATGTTATTC-3'/(R)	365
gyrB	BT1/BT2r	5'-ATCGGTGATACAGATAAGACT-3'/(F) 5'-CCTTCATACGTATGAATATTATTT-3'/(R)	368
cry1Ab	SB-2/U3-18c	5'-TCGGAAAATGTGCCCAT-3'/(F) 5'-AATTGCTTTCATAGGCT-3'/(R)	858
cry1Ac	RB-19/U8-15c	5'-GGGACTGCAGGAGTGAT-3'/(F) 5'-CAGGATTCCATTCAAGG-3'/(R)	653
hblA	HblA1/HblA2	5'-GCTAATGTAGTTTCACCTGTAGCAAC-3'/(F) 5'-AATCATGCCACTGCGTGGACATATAA-3'(R)	873
hblC	HBLC-N/ HBLC-C	5'-AATAGGTACAGATGGAACAGG-3'/(F) 5'-GGCTTTCATCAGGTCATACTC-3'(R)	399
hblD	HBLD-N /HBLD-C	5'-AATCAAGAGCTGTCACGAAT-3'/(F) 5'-CACCA ATTGACCATGCTAAT-3' (R)	439
nheA	nheA344S/ nheB843A	5'-TACGCTAAGGAGGGGCA –3'/(F) 5'-GTTTTTATTGCTTCATCGGCT-3'/(R)	499
bceT	ETF/ ETR	5'-TTACATTACCAGGACGTGCTT-3'/(F) 5'-TGTTTGTGATTGTAATTCAGG-3'/(R)	428
entFM	ENTA /ENTB	5'-ATGAAAAAAGTAATTTGCAGG-3'/(F) 5'-TTAGTATGCTTTTGTGTAACC-3'/(R)	1269
cytK	F2/R7	5'-AACAGATATCGGTCAAAATGC-3'/(F) 5'-CGTGCATCTGTTTCATGAGG-3'/(R)	623

[1]F, forward sequence; R, reverse and complementary sequence. Please confer to Materials and Methods for citations of the primers.

Results and Discussion

Differentiation of *B. cereus, B. thuringiensis,* and *B. mycoides* by PCR and colony morphology PCR with *gyrB* target primer for the differentiation of *B. cereus* group is shown in Fig. 1A and 1B. *gyrB* gene encoding the subunit B protein of DNA gyrase (topoisomerase type II) was used as the target of the primer, and 365- and 368-bp fragments of *gyrB* genes for *B. cereus, B. thuringiensis,* and *B. mycoides* were amplified. PCR assay with BC1/BC2r primer designed specially for the specific detection of *B. cereus* is shown in Fig. 1A. PCR products of 365 bp were shown in *B. cereus* KCCM 40935, KCCM11773, and KCTC 1094, *B. thuringiensis* KCTC 1509 and 3452, whereas none were found in *B. thuringiensis* KCCM 11428, and *B. thuringiensis* subsp. *kurstaki* KCCM 11429. No amplification was observed in *B. mycoides.*

PCR assay with BT1/BT2r primer designed specially for the specific detection of *B. thuringiensis* shown in Fig. 1B. The strains of *B. thuringiensis* KCTC 1509 and 3452 showed expected PCR products of 368 bp, whereas the other strains including *B. thuringiensis* KCCM 11428 and *B. thuringiensis* subsp. *kurstaki* KCCM 11429 did not show the products. To differentiate *B. thuringiensis* strains, PCR with the primers targeted for *cry* genes was also performed for all *B. cereus* group strains (Fig. 1C). The PCR product of 858 bp with SB-2/U3-18c primer designed for *cry1Ab* was shown only in *B. thuringiensis* KCTC 3452, and those of 653 bp with RB-19/U8-15c in *B. thuringiensis* KCCM 11428 and *B. thuringiensis* subsp. *kurstaki* KCCM 11429, which had the PCR products with BC1/BC2r and RB-19/U8-15c primers. There was no amplification with the primers in *B. mycoides.* Therefore, the typical patterns shown in *cry* gene PCR could be used to distinguish *B. thuringiensis* KCTC 3452, *B. thuringiensis* KCCM 11428, *B. thuringiensis* subsp. *kurstaki* KCCM 11429, and *B. cereus,* as well as *B. mycoides.*

Virulence factors (21), restriction fragment length polymorphism (22), pulsed-field gel electrophoresis, analysis of intergenic spacer regions (23), and the arbitrary PCR (18) differentiated *B. anthracis* from *B. cereus,* but failed to differentiate *B. cereus* from *B. thuringiensis* due to the indistinguishable phenotypic and genotypic characteristics of these organisms. However, Manzano *et al.* (17) conducted PCR with *gyrB* gene primers and restriction enzyme digestion, and Cherif *et al.* (24) also attempted to differentiate them by rep-PCR fingerprint typing method. The primers targeted for *gyrB* and *cry* genes, which were developed separately by other groups, were put together here to differentiate *B. cereus* and *B. thuringiensis.*

B. mycoides colony morphology on agar is shown Fig. 2. Typical rhizoid colony morphology, with the long hair or root-like structures, extended several centimeters from inoculation. Rough galaxy-shaped colonies were often produced by *B. cereus* strains and should not be confused with the typical rhizoid growth.

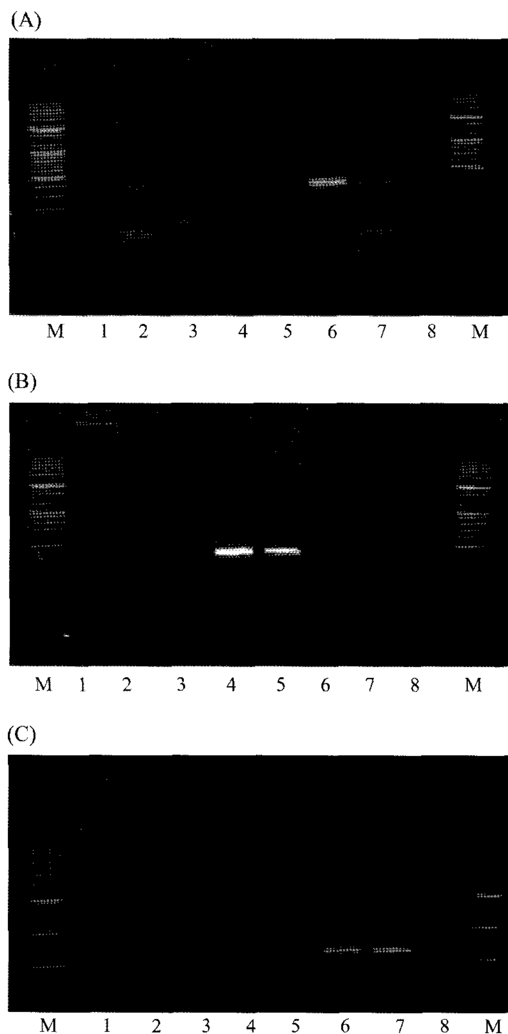

Fig. 1. Agarose gel electrophoresis of *B. cereus* group by PCR with the primers of BC1/BC2r(A), BT1/BT2r(B), SB-2/U3-18c (C), and RB-19/U8-15c(C). M, 100 bp marker; Lane 1, *B. cereus* KCCM 40935; Lane 2, *B. cereus* KCCM 11773; Lane 3, *B. cereus* KCTC 1094; Lane 4, *B. thuringiensis* KCTC 1509; Lane 5, *B. thuringiensis* KCTC 3452; Lane 6, *B. thuringiensis* KCCM 11428; Lane 7, *B. thuringiensis* subsp. *kurstaki* KCCM 11429; Lane 8, *B. mycoides* KCTC 3453. The PCR product of SB-2/U3-18c and RB-19/U8-15c showed at the (C)-5 lane and (C)-6,7 lanes, respectively.

Isolation and identification of *B.cereus* group from the foods by PCR and colony morphology *B. cereus* group strains were isolated from the typical colony from MYP selective media. Because Gram-staining, catalase activity, and hemolysis results revealed *B. cereus* and *B.*

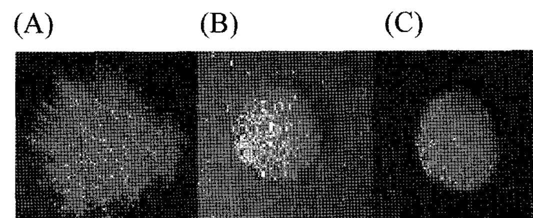

Fig. 2. Colony morphology of rhizoid *B. mycoides* grown on BHI agar in comparison with *B. cereus* and *B. thuringiensis.* (A) *B. mycoides* KCTC 3453; (B) *B. cereus* KCCM 40935; (C) *B. thuringiensis* KCTC 1509.

thurigiensis gave the same characteristics on egg-yolk reaction, mannitol fermentation, and antibiotic degradation, they were differentiated by PCR assays using BC1/BC2r primer for *B. cereus*, and BT1/BT2r, SB-2/U3-18c, and RB-19/U8-15c primers for *B. thuringiensis*. *B. mycoides* was not confirmed by PCR assay; therefore, its rhizoid colony morphology on agar was detected. From 136 samples of 31 unhusked rices, 26 brown rices, 35 Japanese vinegared rice delicacies, and 44 *kimbap*, 66 strains of *B. cereus* group were isolated (Table 2). Again their enterotoxin productions were confirmed using the Oxoid and/or Tecra kits (data not shown).

Among the 66 *B. cereus* group isolates, the numbers of *B. cereus*, *B. thuringiensis*, and *B. mycoides* were 54, 11, and 1, respectively. Among 15 isolates from the boiled rice of Japanese vinegared delicacies, 3 *B. thuringiensis* strains were detected, whereas detection of *B. mycoides* was low in the MYP media. Most isolates were *B. cereus* strains; however, *B. thuringiensis* was detected in about 10% of the edible samples except the unhusked rice. Wei *et al.* (25) reported that 49.8% of the ready-to-eat foods was contaminated with *B. cereus* in Taiwan, whereas Sarrias *et al.* (15) isolated *B. cereus* from almost all raw Spanish rice samples tested. In this study, about 50% prevalence was confirmed for the ready-to-eat rice products. In addition, contamination of *B. thuringiensis* was confirmed in the rice and its processed products. These results indicate *B. thuringiensis* as well as *B. cereus* will be a food-borne pathogen.

Detection of enterotoxin genes of *B. cereus* group by PCR To confirm the distribution of toxin genes on *B. cereus, B. thuringiensis,* and *B. mycoides*, PCR assays for the detection of hemolysin BL gene and non-hemolytic enterotoxin gene were performed (Table 3). Primers HblA1/HblA2 were designed for the specific detection of *hblA* gene encoding B component of HBL in *B. cereus* group (19). Seven of eight *B. cereus* group type strains had

Table 2. Prevalence of *B. cereus* group isolated from raw rice and its processed products in Korea

Sources	Sample No.	Isolated number of *B. cereus* group		
		B. cereus	*B. thuringiensis*	*B. mycoides*
Unhusked rice	31	9 (29.0[1], 56.3[2])	6 (19.4, 37.5)	1 (3.2, 6.3)
Brown rice (raw)	26	13 (50.0, 92.9)	1 (3.8, 7.1)	-
Vinegared rice delicacies (Boiled rice)	35	15 (42.9, 83.3)	3 (8.6, 7.0)	-
Kimbab	44	17 (38.6, 94.4)	1 (2.3, 5.6)	-
Total	136	54 (39.7, 81.8)	11 (8.1, 16.7)	1 (0.7, 1.5)

[1]Number indicated the prevalence (%) of each species among the samples tested.
[2]Number indicated the relative ratio(%) of each species among the *B. cereus* group isolated from each sample.

Table 3. Detection of the enterotoxin genes of *B. cereus* group by polymerase chain reaction

Strains	*hbl*			*nheA*	*cytK*	*bceT*	*entFM*
	hblA	*hblC*	*hblD*				
B. cereus KCCM 40935	+[1]	+	+	+	-	+	+
B. cereus KCCM11773	-	-	-	+	-	-	+
B. cereus KCTC 1094	+	+	+	+	+	+	+
B. cereus B-38B	+	+	+	+	+	+	+
B. cereus B-50B	+	+	+	+	+	+	+
B. thuringiensis KCTC 1509	+	+	+	+	+	+	+
B. thuringiensis KCTC 3452	+	+	+	+	-	-	+
B. thuringiensis KCCM 11428	+	+	+	+	+	+	+
B. thuringiensis KCCM 11429	+	+	+	+	+	+	+
B. thuringiensis B-28A	+	-	-	-	-	-	+
B. thuringiensis B-34B	+	+	+	+	-	-	+
B. mycoides KCTC 3453	+	+	+	-	-	+	-
B. mycoides B-59	-	+	+	-	-	+	+
B. mycoides 11-2	-	+	+	-	-	+	+

[1]+, a PCR product of the expected size was seen; -, no PCR product was formed.

PCR products of 873 bp for hemolysin BL gene; however, *B. cereus* KCCM 11773 and *B. mycoides* isolates showed no products. PCR products of *hblC* and *hblD* genes were obtained from all *B. cereus* group strains except *B. cereus* KCCM 11773 and *B. thuringiensis* B-28A. Others also found that 43% of *B. cereus* and all *B. thuringiensis* type strains tested had the *hblA* gene (28). Mäntynen *et al.* (19) detected *hblA* in 52% of *B. cereus* strains and in one *B. thuringiensis* strain, and found these strains to be enterotoxin-positive as revealed using Oxoid kit. Hsieh *et al.* (14) found that all 36 *hblA* gene PCR-positive strains, including 26 *B. cereus*, 8 *B. thuringiensis*, and 2 *B. mycoides* strains, also produced enterotoxins (15). Therefore, *hbl* gene of hemolysin BL enterotoxin, consisting of *hblA, hblC*, and *hblD* operons, was broadly found, ranging from 52 to 100%, in *B. cereus* and *B. thuringiensis*.

PCR assay with nheA344S/nhe843 primer for the detection of *nheA* gene is shown in Table 3. All 5 *B. cereus* strains tested and 5 *B. thuringiensis* among six strains, whereas none of the *B. mycoides* strains, were *nheA* PCR-positive. Bjarne *et al.* (27) detected *nheA* in 33 of the 41 *B. thuringiensis* strains, again a significantly higher proportion among the *B. cereus* group. Using F2/ R7 primer *cytK* gene was found in 3 each *B. cereus* and *B. thuringiensis* strains, while none in *B. mycoides* strains. PCR assays using ETF/ ETR primer were designed for enterotoxin T gene, and PCR products of 428 bp were found in 4 *B. cereus* and 3 *B. thuringiensis* strains, and none in *B. mycoides* strains. Asano *et al.* (5) isolated the gene in 41 of 84 *B. cereus* strains, 2 of 3 *B. mycoides* strains, and 8 of 9 *B. thuringiensis* strains examined.

The 45-kDa enterotoxin (EntFM) isolated from *B. cereus* strain FM-1 has been shown to be cytotoxic to the vero cells, although no hemolytic activity has been detected (28). PCR assays using ENTA/ENTB primer were designed for the detection of *entFM* gene, and all *B. cereus* group strains tested except one *B. mycoides* showed products of 1269 bp. Hsieh *et al.* (14) found the gene from 78 of 84 *B. cereus* strains, 1 of 3 *B. mycoides* strains, and 7 of 9 *B. thuringiensis* strains examined. In addition, 27 of 28 food isolates and all 30 outbreak-associated strains were *entFM* PCR-positive. Thus, *entFM* gene was found to be the most prevalent enterotoxin gene in *B. cereus*.

Therefore, the five enterotoxin genes appear to be distributed broadly in all *B. cereus* group strains, particularly in *B.cereus* and *B.thuringiensis*, suggesting *B. thuringiensis* as well as *B. cereus*, which produce the toxins, are potential vehicles for *B. cereus*-related food-borne outbreaks.

Considering the high prevalence of *B. cereus* group in rice and broad enterotoxin distribution, it is reasonable to regulate ready-to-eat rice products based on enterotoxin production. However, further researches are needed for quantitative contamination analysis and to confirm which toxin is the most powerful poisoning agent.

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References

1. Grethe IB, Andersen S, Marianne S, Terje LT, Einar GT. Growth and toxin profiles of *Bacillus cereus* isolated from different food sources. Int. J. Food Microbiol. 69: 237-246 (2001)
2. Notermans S, Batt CA. A risk assessment approach for food borne *Bacillus cereus* and its toxins. J. Appl. Microbiol. Symp. Suppl. 84: 51S-61S (1998)
3. Agata N, Masashi M, Ohta M, Suwan S, Ohtani I, Isobe M. A novel dodecadepsipeptide, cereulide, isolated from *Bacillus cereus* causes vacuole formation in Hep-2 cells. FEMS Microbiol. Lett. 121: 31-34 (1994)
4. Agata N, Ohta M, Arakawa Y, Mori M. The *bceT* gene of *Bacillus cereus* encodes an enterotoxin protein. Microbiology 141: 983-988 (1995)
5. Asano SI, Nukumizu Y, Bando H, Hzuka T, Yamamoto T. Cloning of novel enterotoxin genes from *Bacillus cereus* and *Bacillus thuringiensis*. Appl. Environ. Microbiol. 63: 1054-1057 (1997)
6. Lund T, De Buyser ML, Granum PE. A new cytotoxin from *Bacillus cereus* that may cause necrotic enteritis. Mol. Microbiol. 38: 254-261 (2000)
7. Beecher DJ, Shoeni JL, Wong ACL. Enterotoxin activity of hemolysin BL from *Bacillus cereus*. Infect. Immun. 63: 4423-4428 (1995)
8. Lund T, Granum PE. Characterisation of non-haemolytic enterotoxin complex from *Baciilus cereus* isolated after a foodborne outbreak. FEMS Microbial Lett. 141: 151-156 (1996)
9. Manuel P, Victor JP. PCR-based identification of *Bacillus thuringiensis* pesticidal crystal genes. FEMS Microbiol. Review 26: 419-432 (2003)
10. Buyer JS. A soil and rhizosphere microorganism isolation and enumeration medium that inhibits *Bacillus mycoides*. Appl. Environ. Microbiol. 61: 1839-1842 (1995)
11. Ash C, Farrow JA, Dorsch M, Stackebrandt E, Collins MD. Comparative analysis of *Bacillus anthracis, Bacillus cereus,* and related species on the basis of reverse transcriptase sequencing of 16S rRNA. Int. J. Syst. Bacteriol. 41: 343-346 (1991)
12. Fletcher P, Logan E. Improved cytotoxicity assay for *Bacillus cereus* diarrhoeal enterotoxin. Lett. Appl. Microbiol. 28: 394-400 (1999)
13. Rivera AMG, Granum PE, Priest FG. Common occurrence of enterotoxin genes and enterotoxicity in *Bacillus thuringiensis*. FEMS Microbiol Lett. 190: 151-155 (2000)
14. Hsieh YM, Sheu SJ, Chen YL, Tsen HY. Enterotoxigenic profiles and polymerase chain reaction detection of *Bacillus cereus* group cells and *B. cereus* strains from foods and food-borne outbreaks. J. Appl. Microbiol. 87: 481-490 (1994)
15. Sarrias JA, Valero M. Salmeron MC. Enumeration, isolation and characterization of *Bacillus cereus* strains from Spanish raw rice. Food Microbiol. 19: 589-595 (2002)
16. Lee JH, Kim M, Um S. PCR-based detection and identification of *Lactobacillus plantarum, Lactobacillus pentosus*, and *Lactobacillus paraplantarum* in kimchi. Food Sci. Biotechnol. 13: 754-757 (2004)
17. Manzano M, Cocolin L, Cantonic C. Comi G. *Bacillus cereus, Bacillus thuringiensis* and *Bacillus mycoides* differentiation using a PCR-RE technique. Int. J. Food Microbiol. 81: 249-254 (2003)
18. Bourque SN, Valero JR, Mercier J, Lavoie MC, Levesque KJ. Multiplex polymerase chain reaction for detection and differentiation of the microbial insecticide *Bacillus thuringienesis*. Appl. Environ. Microbiol. 59: 523-527 (1993)
19. Mäntynen V, Lindström KA. Rapid PCR-based DNA test for enterotoxic *Bacillus cereus*. Appl. Environ. Microbiol. 64: 1634-1639 (1998)
20. Granum PE, O'Sullivanand K, Lund T. The sequence of the non-hemolytic enterotoxin operon from *Bacillus cereus*. FEMS Microbiol. Lett. 177: 225-229 (1999)
21. Henderson I, Yu D, Turnbull PC. Differentiation of *Bacillus anthracis* and other '*Bacillus cereus* group' bacteria using IS231-derived sequences. FEMS Microbiol. Lett. 128: 113-118 (1995)
22. Keim P, Kalif A, Schupp P, Hill JK, Travis SE, Richmond K, Adair DM, Hugh-Jones M, Kuske CR, Jackson P. Molecular evolution

and diversity in *Bacillus anthracis* as detected by amplified fragment length polymorphism markers. J. Bacteriol. 179: 818-824 (1997)

23. Harrell LJ, Andersen GL, Wilson KH. Genetic variability of *Bacillus anthracis* and related species. J. Clin. Microbiol. 33: 1847-1850 (1995)
24. Cherif A, Brusetti L, Borin S, Rizzi A, Boudabous A, Khyami-Horani H. Genetic relationship in the *Bacillus cereus* group by rep-PCR fingerprinting and sequencing of a *Bacillus anthracis*-specific rep-PCR fragment. J. Appl. Microbiol. 94: 1108-1119 (2003)
25. Wei QR, Liao MJ, Hung MJ, Wan TH. Microbiological quality of 18 degreeCready-to-eat food products sold in Taiwan. Int. J. Food Microbiol. 80: 241-250 (2003)
26. Birgit MP, Richard D, Birgit D, Märtlbauer E, Siegfried S. The hemolytic enterotoxin HBL is broadly distributed among species of the *Bacillus cereus* group. Appl. Environ. Microbiol. 65: 5436-5442 (1999).
27. Bjarne MH, Hendriksen NB. Detection of enterotoxic *Bacillus cereus* and *Bacillus thuringiensis* Strains by PCR Analysis. Appl. Environ. Microbiol. 67: 185-189 (2001)
28. Shinagawa K, Sugiyama J, Terada T, Matsusaka N, Sugii S. Improved methods for purification of an enterotoxin produced by *Bacillus cereus*. FEMS Microbiol. Lett. 64: 1-5 (1991)