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Evaluation of Various PCR Assays for Detection of Emetic-Toxin-Producing *Bacillus cereus*

Kim, Jung-Beom[1], Jae-Myung Kim[2], Yong-Bae Park[1], Jeong-A Han[3], Soon-Ho Lee[3], Hyo-Sun Kwak[3], In-Gyun Hwang[3], Mi-Hye Yoon[1], Jong-Bok Lee[1], and Deog-Hwan Oh[2]*

[1]*Division of Health Research and Planning, Gyeonggi-do Research Institute of Health and Environment, Suwon 440-290, Korea*
[2]*School of Bioscience and Biotechnology, Kangwon National University, Chuncheon 200-701, Korea*
[3]*Food Microbiology Division, Korea Food and Drug Administration, Seoul 122-704, Korea*

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Because conventional methods for detecting emetic-toxin-producing *B. cereus* are laborious and costly, various PCR assays, which are easy and cheap, have recently been reported. Therefore, this study estimated and compared the ability of various PCR assays to detect emetic-toxin-producing *B. cereus* strains isolated in Korea. The PCR assays were performed on 160 *B. cereus* strains, including 40 emetic-toxin-producing strains. Although the species-specific PCR assays were all shown to be highly specific, the sensitivities varied greatly. The accuracies of the primers were 97.5% (CER), 95.6% (EM1), 96.3% (RE234), 89.4% (CES), and 83.1% (Ces3R/CESR2). Moreover, the CER primer had a higher sensitivity (100%) than all the other primers tested, and a specificity of 96.7%. Thus, the CER primer was shown to be the most effective for screening the emetic-toxin-producing *B. cereus* strains tested in this study. However, the ability of these PCR assays to identify emetic-toxin-producing *B. cereus* should also be confirmed using other methods.

Keywords: *Bacillus cereus*, PCR assay, cereulide, emetic toxin

Bacillus cereus is a Gram-positive, rod-shaped, and spore-forming food pathogen that can cause diarrheal- and emetic-type food poisoning [3], and is commonly found in a wide variety of different foods and environments [4, 7]. The enterotoxins that have been associated with diarrheal-type food poisoning are hemolysin BL (HBL), non-hemolytic enterotoxin (NHE), cytotoxin K (CytK), and enterotoxin FM [20, 27]. In addition, the symptoms of diarrheal-type food poisoning, including abdominal pain and watery diarrhea are similar to those of *Clostridium perfringens* [10] and occur 8–16 h after the ingestion of contaminated food [17, 18]. Enterotoxins have already been comparatively well characterized at the molecular level [20, 24] and in immunochemical assays [5, 6].

Emetic-type food poisoning is caused by an emetic toxin (cereulide), a small, heat and acid stable circular dodecadepsipeptide with the following stereochemistry: [D-*O*-Leu-D-Ala-L-*O*-Val-L-Val]$_3$ [9]. This type of food poisoning is characterized by nausea, vomiting, and abdominal pain occurring only 1–5 h after the consumption of contaminated food, such as fried rice and cooked rice [1, 21]. Emetic-type food poisoning is predominant in Japan [19], and the dietary life of Koreans is very similar to that in Japan. Thus, the potential risk of emetic-type food poisoning caused by *B. cereus* also exists in Korea. However, only one emetic outbreak [16] was reported in 2008 among 27 outbreaks associated with *B. cereus* from 2001 to 2008 in Korea (http://fm.kfda.go.kr). Therefore, this may indicate that emetic-type food poisoning is being underestimated, as the symptoms are generally temporary and relatively mild, not requiring medical attention [3], plus the symptoms are similar to those for staphyloenterotoxicosis [25]. However, an immunochemical assay for detecting an emetic toxin, characterized as poorly antigenic, has not yet been developed [28], and available methods for detecting emetic-toxin-producing *B. cereus* are typically laborious and costly, such as a boar sperm assay, rat liver mitochondria assay, Hep-2 cell culture assay, and HPLC/MS analysis [3, 14, 15].

Notwithstanding, PCR assays, which are easy and cheap, have recently been developed to detect emetic toxin synthesis-related genes [8, 9, 13, 23, 26]. Weber and Marahiel [30] reported that important bioactive peptides, such as β-lactam antibiotics and cyclosporine, are synthesized *via* a nonribosomal mechanism employing nonribosomal peptide

**Corresponding author*
Phone: +82-33-250-6457; Fax: +82-33-241-0508;
E-mail: deoghwa@kangwon.ac.kr

synthetase (NRPS). Thus, a high correlation between the presence of the NRPS gene and emetic toxin production is suggested based on the heterocyclic structure of an emetic toxin and the presence of D-amino acids in the peptides [8, 13, 29]. The first PCR assay, the EM1 primer pair, for the detection of emetic strains was reported in 2004 based on the NRPS responsible for emetic toxin synthesis [8]. The CER primer pair was then designed based on conserved regions of the NRPS gene [13], whereas the RE234 primer pair was suggested based on a sequence-characterized amplified region derived from an amplified polymorphic DNA (RAPD) fragment [23]. Ehling-Schulz *et al*. [9] reported on a DNA fragment from an emetic-toxin-producing *B. cereus*, where the putative gene product showed a significant homology to valine activation NRPS modules. As such, the CES primer pair was developed based on the valine activation NRPS modules. Therefore, the comparative effectiveness of these PCR assays needs to be evaluated to determine whether they can correctly detect Korean emetic-toxin-producing *B. cereus* isolates, and to prevent misdiagnosis between *Staphylococcus aureus* and *B. cereus* food poisoning.

Accordingly, this study estimated and compared the ability of various PCR assays to detect emetic-toxin-producing *B. cereus* strains isolated from clinical samples, grains, and food samples in Korea.

Materials and Methods

Bacterial Strains

A total of 160 *B. cereus* strains isolated from clinical samples (81 strains), grains (65 strains), and food samples (14 strains) were used in this study (Table 1). Among the 81 clinical *B. cereus* isolates, 23 strains were obtained from the Jeollabuk-do Research Institute of Health and Environment (JNHE), 13 isolates from the National Institute of Health (KNIH), 9 isolates from the Incheon Metropolitan City Research Institute of Health and Environment (ICHE), 6 isolates from the Seoul Metropolitan City Research Institute of Health and Environment (SEHE), 24 isolates from the Gyeonggi-do Research Institute of Health and Environment (GIHE), and 6 stock strains from the Department of Food Science and Biotechnology, Kangwon National University, Korea. The 65 strains from grains were obtained from GIHE (64 strains) and KUGH (1 strain), and the 14 strains from foods samples were obtained from GIHE (11 strains), KUGH (1 strain), and the Korea Food and Drug Administration (KFDA, 2 strains). The emetic-toxin-producing reference strain used for the positive control was *B. cereus* F4810/72 and the diarrheal-toxin-producing reference strains used for the negative control were *B. cereus* ATCC 11778, ATCC 21772, and ATCC 14579.

Table 1. *Bacillus cereus* strains used in this study.

Origin	No. of *B. cereus* strains		
	Emetic-toxin-producing strains[a]	Diarreal-toxin-producing strains	Total
Clinical strains	36	45	81
Grain strains	1	64	65
Food strains	3	11	14
Total	40	120	160

[a]HPLC/MS with an ion-trap detector was performed for the detection of emetic-toxin-producing *B. cereus.*

Assay for Detecting Emetic-Toxin-Producing *B. cereus*

HPLC/MS analysis was performed to detect the emetic-toxin-producing strains from 160 *B. cereus* isolates. A *Bacillus* Diarrhoeal Enterotoxin Visual Immunoassay (BDE−VIA) kit (Tecra International Pty Ltd., NSW, Australia) and *B. cereus* Enterotoxin-Reversed Passive Latex Agglutination (BCET−RPLA) kit (Oxoid, Hampshire, U.K.) were used to detect diarrheal toxins, such as non-hemolytic enterotoxin (NHE) and hemolysin BL (HBL) enterotoxin in emetic-toxin-nonproducing *B. cereus* strains. Both kits were used according to the manufacturers' instructions. The emetic toxin was extracted according to previous reports with a minor modification [12, 14]. Two or three colonies of *B. cereus* grown at 28°C for 4 days on a tryptone soya agar (TSA, Oxoid, England) were lysed based on three freeze−thaw cycles and extracted with 200 µl of methanol overnight. The filtered (0.45 µm) extract was used as the samples for the HPLC/MS analysis, which was carried out according to a previous report with a minor modification [2]. The HPLC/MS analysis was performed with an ULTRA 3000 HPLC (Dionex, U.S.A.) equipped with an ESI electrospray ion-trap mass analyzer (LCQ Advantage Max; Thermofinigan, U.S.A.) using a XeHera C_{18} column (100 by 2.1 mm, 3-µm particle size). The mobile phase consisting of 95% (v/v) acetonitrile, 4.9% (v/v) H_2O, and 0.1% (v/v) trifluoroacetic acid was injected at a flow rate of 0.25 ml/min. The A_{258} was monitored using a UV detector, and the source parameters were as follows: capillary, −3,700 V; end-point offset, −500 V; Nebulizer, 40.0 ml/min; dry gas, 7.0 l/min; and dry temperature, 300°C. The sample injection volume was 5.0 µl and values were collected for the mass spectrum from 1,100 to 1,200 *m/z*. The *m/z* values specific for molecular emetic toxin ions were 1,153 ($M+H^+$ adduct), 1,170 ($M+NH_4^+$ adduct), and 1,191 ($M+K^+$ adduct). *B. cereus* F4810/72 was used as the reference strain.

DNA Extraction

All the *B. cereus* strains were grown on TSA plates at 35°C for 24 h. A single colony was inoculated into Luria−Bertani (LB; Difco, Detroit, MI, U.S.A.) broth and incubated at 35°C for 8 h. One ml of the cell suspension was then harvested and centrifuged at 13,000 ×*g* for 5 min at 4°C. The supernatants were removed and the pellet was resuspended in 1 ml of sterile distilled water. After a second round of centrifugation under the same conditions, the pellet was resuspended in 500 µl of sterile distilled water, boiled for 10 min, and centrifuged at 13,000 ×*g* for 5 min at 4°C. The DNA concentration was determined using a spectrophotometer at A_{260}. The supernatants were stored at −20°C until used as templates for the different PCR assays.

PCR Assays for Detection of Emetic-Toxin-Producing *B. cereus*

To compare the ability of each PCR assay to detect emetic-toxin-producing *B. cereus*, the primer pairs and PCR reaction conditions used in this study are shown in Table 2. The PCR reaction was performed in a thermal cycler (Mastercycler Gradient S; Eppendrop, Germany) using 20-µl reaction volumes containing 10 mM Tris-HCl,

Table 2. PCR primers and cycling conditions for the detection of emetic-toxin-producing *Bacillus cereus*.

Primer name	Primer sequence (5'–3')	PCR cycling conditions	Amplicon size (bp)	Reference
CER	ATCATAAAGGTGCGAACAAGA AAGATCAACCGAATGCAACTG	95°C, 10 min→(94°C, 60 sec→52°C, 60 sec→72°C, 60 sec) 35 cycles→72°C, 5 min	188	[13]
EM1	GACAAGAGAAATTTCTACGAGCAAGTACAAT GCAGCCTTCCAATTACTCCTTCTGCCACAGT	95°C, 15 min→(95°C, 30 sec→60°C, 30 sec→72°C, 60 sec) 30 cycles→72°C, 5 min	635	[8]
RE234	AACGTCGGTATGATTTTAGG CTCTTCTGCTCTCTATTTATGTC	95°C, 1 min→(95°C, 5 sec→54°C, 10 sec→72°C, 20 sec) 35 cycles→72°C, 5 min	234	[23]
CES	GGTGACACATTATCATATAAGGTG GTAAGCGAACCTGTCTGTAACAACA	95°C, 15 min→(95°C, 60 sec→53°C, 75 sec→72°C, 50 sec) 5 cycles→(95°C, 60 sec→58°C, 75 sec→72°C, 50 sec) 25 cycles→72°C, 5 min	1,271	[9]
Ces3R /CESR2	TTGTTGGAATTGTCGCAGAG GTAAGCGAACCTGTCTGTAACAACA	95°C, 3 min→(95°C, 30 sec 60°C, 30 sec→72°C, 60 sec) 35 cycles→72°C, 5 min	405	[26]

1.5 mM $MgCl_2$, 250 μM dNTP, each primer 1 pM, 2 U *Taq* polymerase (Takara *Taq*, Japan), and 2 μl of template DNA. Each PCR reaction was conducted in triplicate. The amplified products were separated by electrophoresis on a 2% agarose gel in 0.5× TBE buffer. The gels were stained with ethidium bromide and visualized using a UV transilluminator (Gel Doc 2000; Bio-Rad, Hercules, CA, U.S.A.).

Validation of Different PCR Assays

The sensitivity, specificity, and accuracy of the different PCR assays were determined by comparing the detection results obtained from each PCR assay with the results of an HPLC/MS analysis for the detection of emetic-toxin-producing *B. cereus*. The sensitivity was defined as the true positive rate percentage calculated based on the number of true positives divided by the sum of true positives plus the false negatives. Sensitivity indicates the efficiency of an assay in detecting emetic-toxin-producing *B. cereus* [11]. Meanwhile, the specificity was defined as the true negative rate percentage calculated based on the number of true negatives divided by the sum of false positives plus the true negatives. Specificity expresses the efficiency of an assay in correctly excluding diarrheal-toxin-producing *B. cereus* [11]. Finally, the accuracy was defined as the correct result rate percentage calculated based on the sum of true positives plus the true negatives divided by the total number of strains tested. Accuracy is a measure of the efficiency of an assay in correctly classifying emetic-toxin- and diarrheal-toxin-producing *B. cereus* [11].

RESULTS AND DISCUSSION

Confirmation of Emetic-Toxin-Producing *B. cereus* Strains Using HPLC/MS and PCR Reaction Mixture

The emetic-toxin-producing ability of 160 *B. cereus* strains collected from clinical samples, grains, and food samples was analyzed using HPLC/MS with an ion-trap mass analyzer. As a result, 40 *B. cereus* strains (36 clinical, 1 grain, and 3 food strains) were identified as emetic-toxin-producing strains owing to the presence of HPLC/MS ion ranges (*m/z*) of 1,153 ($M+H^+$ adduct), 1,170 ($M+NH_4^+$ adduct), and 1,191 ($M+K^+$ adduct), as observed in the *B. cereus* F4810/72 emetic-toxin-producing reference strain (Table 3 and Fig. 1) and previous studies [2, 22]. BDE–

Table 3. The results obtained from different PCR assays for the detection of emetic-toxin-producing *Bacillus cereus*.

	Strain No.	Source	Emetic-toxin production[a]	Emetic-toxin-producing *B. cereus* specific PCR primers				
				CER	EM1	RE234	CES	Ces3R/CESR2
Group I	JNHE 6	Feces	+	+	+	-	+	+
	JNHE 7	Feces	+	+	-	+	-	-
	JNHE 13	Feces	+	+	+	+	-	+
	JNHE 15	Feces	+	+	+	+	-	+
	JNHE 21	Feces	+	+	+	+	+	-
	JNHE 22	Feces	+	+	+	+	-	-
	JNHE 23	Feces	+	+	+	+	-	-
	JNHE 24	Feces	+	+	+	+	+	-
	JNHE 41	Feces	+	+	+	+	+	-
	JNHE 53	Feces	+	+	+	+	+	-

Table 3. Continued.

	Strain No.	Source	Emetic-toxin production[a]	Emetic-toxin-producing *B. cereus* specific PCR primers				
				CER	EM1	RE234	CES	Ces3R/CESR2
Group I	JNHE 54	Feces	+	+	+	+	+	-
	JNHE 56	Feces	+	+	+	+	+	-
	JNHE 60	Feces	+	+	+	+	+	-
	JNHE 61	Feces	+	+	+	+	+	-
	JNHE 78	Feces	+	+	+	+	+	+
	JNHE 80	Feces	+	+	-	+	+	-
	JNHE 82	Feces	+	+	+	+	+	-
	JNHE 88	Feces	+	+	+	+	+	+
	JNHE 95	Feces	+	+	-	+	-	+
	KNIH 20	Feces	+	+	+	+	-	-
	KNIH 24	Feces	+	+	+	+	-	+
	KNIH 25	Feces	+	+	+	+	+	+
	KNIH uls 1	Feces	+	+	+	+	-	+
	KNIH uls 2	Feces	+	+	+	-	-	-
	KNIH uls 3	Feces	+	+	+	+	+	-
	KNIH uls 4	Feces	+	+	+	+	-	+
	KNIH uls 5	Feces	+	+	+	+	-	-
	KNIH uls 6	Feces	+	+	+	+	-	-
	KNIH uls 7	Feces	+	+	+	+	-	-
	KNIH uls 8	Feces	+	+	+	+	-	-
	KUGH 164	Feces	+	+	+	+	+	-
	KFDA 229	Food	+	+	+	+	-	+
	KFDA 250	Food	+	+	-	+	+	+
	KUGH 27	Food	+	+	+	+	+	-
	KUGH 85	Food	+	+	+	+	+	-
	KUGH 10	Vomit	+	+	+	+	+	-
	KUGH 11	Vomit	+	+	+	+	+	-
	KUGH 12	Vomit	+	+	+	+	+	+
	JNHE 36	Feces	+	+	-	-	+	-
	KNIH 28	Feces	+	+	-	-	-	+
Group II	JNHE 38	Feces	-	+	-	-	-	-
	KNIH 29	Feces	-	+	-	-	-	+
	KUGH 143	Feces	-	+	-	+	-	-
	KUGH 13	Vomit	-	+	+	+	-	-
Group III	Clinical strains[b] (n=41)	Feces	-	-	-	-	-	-
	Grain strains[c] (n=64)	Grain	-	-	-	-	-	-
	Food strains[d] (n=11)	Food	-	-	-	-	-	-

[a]HPLC/MS with an ion-trap detector was performed to detect emetic toxin.
[b]Clinical strains contained GIHE 5609, 5616, 5618, 5619, 5625, 5629, 5633, 5674~ 5677, 5679~5689, 6011, 6013; ICHE 0901~0909, SEHE 4153, 4154, 4158, 4165, 4300, 4303; and JNHE 63, 66.
[c]Grain strains contained GIHE 5603, 5605, 5610~5615, 5620, 5621, 5623, 5624, 5626~5628, 5630, 5632, 5634~5668, 5670~5673, 5691~5698.
[d]Food strains contained GIHE 5617, 6007, 6008, 5602, 5607, 5601, 4605, 4616, 4618, 4628, 4629.

VIA and BCET–RPLA kits were also used to detect NHE and HBL enterotoxins among the remaining emetic-toxin-nonproducing strains (120 strains). All strains showed NHE or HBL enterotoxin, except 6 strains that carried at least one of the *hbl*, *nhe*, *cytK*, and *entFM* enterotoxin genes (data not shown). The 120 emetic-toxin-nonproducing strains produced and possessed enterotoxins or enterotoxin genes. All the strains used in this study were then divided

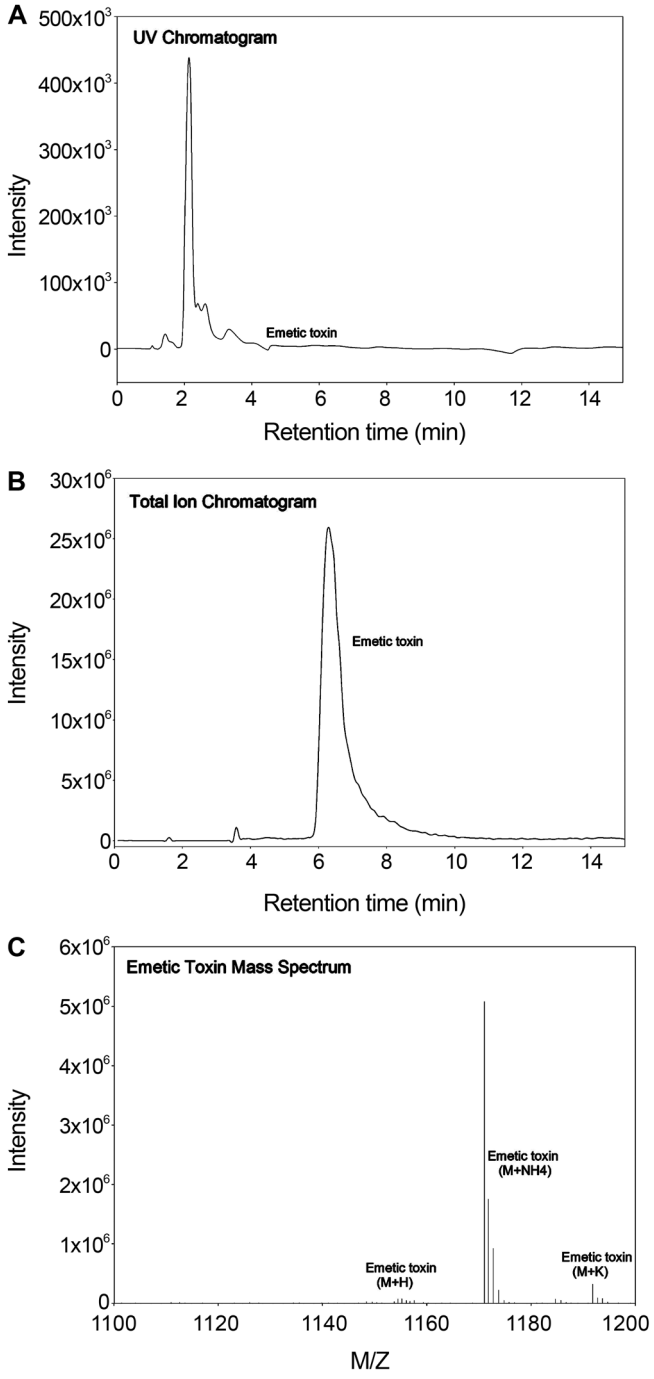

Fig. 1. HPLC/MS analysis of emetic toxin from methanol extracts of tryptone soya agar cultures of *Bacillus cereus* F4810/72 as reference strain.
UV chromatogram (**A**), total ion chromatogram (**B**), and emetic toxin mass spectrum (**C**).

into two groups, emetic-toxin-producing (n=40) and diarrheal-toxin-producing strains (n=120), based on the HPLC/MS analysis results.

As PCR assays utilize different reaction volumes and concentrations of $MgCl_2$, dNTP, and the buffer, the same PCR reaction mixture was used to evaluate 5 different PCR primers and reaction conditions with 3 diarrheal-toxin-producing reference strains (*B. cereus* ATCC 14579, ATCC 21772, and ATCC 11778) and one emetic-toxin-producing reference strain (*B. cereus* F4810/72) (Fig. 2). All PCR assays showed that each amplicon was related to the emetic toxin synthesis genes in *B. cereus* F4810/72, as previously reported [8, 9, 13, 23, 26], and no PCR product was observed for the 3 diarrheal-toxin-producing reference strains.

Evaluation of Different PCR Assays for Detection of Emetic-Toxin-Producing *B. cereus*

A variety of different bioactive peptides, such as β-lactam antibiotics and cyclosporine, are synthesized *via* a nonribosomal mechanism employing large enzyme complexes known as NRPS [30]. The heterocyclic structure of an emetic toxin and the presence of D-amino acids in such peptides may indicate a correlation between the presence of the NRPS gene and emetic toxin production [13, 29]. Thus, PCR assays for detecting emetic toxin producing *B. cereus* have been developed based on NRPS, which is responsible for emetic toxin synthesis [8, 9, 13, 26]. Therefore, all 160 *B. cereus* strains, including the 40 emetic-toxin-producing strains confirmed by the HPLC/MS analysis, were subjected to these PCR assays developed for detecting the emetic-toxin-synthesis-related genes (Table 3), and the strains grouped according to the results of the HPLC/MS analysis and PCR assays. Group I consisted of the 40 strains that were positive in the HPLC/MS analysis and most of the PCR assays. Group II contained 4 strains that were positive in the PCR assays, yet negative in the HPLC/MS analysis. Group III consisted of strains that were neither positive in the PCR assays nor in the HPLC/MS analysis (n=116 strains).

Although the 5 species-specific PCR assays were shown to be highly specific, the sensitivity of the assays varied greatly (Table 4). The CER primer, developed based on the conserved regions of the NRPS gene [13], showed a higher sensitivity (100%) than all the other primers tested in this study. The EM1 primer, designed to target the conserved sequence motifs of the NRPS genes [8], exhibited a sensitivity of 85.0% for emetic-toxin-producing *B. cereus*, yet produced false-negative results for 6 diarrheal-toxin-producing *B. cereus*. The RE234 primer, designed based on a sequence-characterized amplified region derived from an amplified polymorphic DNA (RAPD) fragment, showed a sensitivity of 90.0% for emetic-toxin-producing *B. cereus*, yet produced false-negative results for 4 diarrheal-toxin-producing *B. cereus* [23]. The CES primer, developed based on a DNA fragment, where the putative gene product from an emetic-toxin-producing *B. cereus* shows a significant homology to valine activation NRPS modules [9], exhibited a sensitivity of 57.5%, and the Ces3R/CESR2 primer,

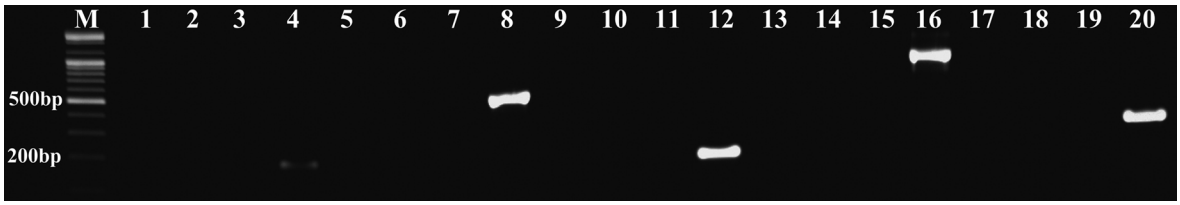

Fig. 2. PCR analysis of emetic toxin synthesis-related genes of *B. cereus* reference strains using CER (lanes 1–4), EM1 (lanes 5–8), RE234 (lanes 9–12), CES (lanes 13–16), and Ces3R/CESR2 (lanes 17–20) primers.

Table 4. Comparison of the detection of emetic-toxin-producing *Bacillus cereus* strains between the PCR assays and HPLC/MS analysis.

PCR primers	Emetic-toxin-producing *B. cereus* strains (n=40)		Diarrheal-toxin-producing *B. cereus* strains (n=120)		Sensitivity[a] (%)	Specificity[b] (%)	Accuracy[c] (%)
	Detection of emetic toxin related gene amplicon (a)	Non-detection of emetic toxin related gene amplicon (b)	Detection of emetic toxin related gene amplicon (c)	Non-detection of emetic toxin related gene amplicon (d)			
CER	40	0	4	116	100.0	96.7	97.5
EM1	34	6	1	119	85.0	99.2	95.6
RE234	36	4	2	118	90.0	98.3	96.3
CES	23	17	0	120	57.5	100.0	89.4
Ces3R/CESR2	14	26	1	119	35.0	99.2	83.1

[a]Sensitivity was calculated by the number of true positives (a) divided by the sum of true positives (a) plus false negatives (b).
[b]Specificity was calculated by the number of true negatives (d) divided by the sum of false positives (c) plus true negatives (d).
[c]Accuracy was calculated by the sum of true positives (a) plus true negatives (d) divided by the number of total strains tested.

redesigned based on the CES primer [26], showed a sensitivity of 35.0%. This variety in the sensitivity of the different PCR assays occurred because each primer is designed to target different regions of the genes and RAPD fragments related to emetic toxin production. However, very little work has yet been done to develop species-specific primers for genes related to emetic toxin production by Korean emetic-toxin-producing *B. cereus* strains.

The CES primer, designed based on the valine activation NRPS modules responsible for emetic toxin production [9], had a specificity of 100%. Meanwhile, the CER primer had a specificity of 96.7%, which was the lowest specificity among all the primers tested in this study. The CES primer produced false-positive results for JNHE 38, KNIH 29, and KUGH 143, isolated from diarrheal patient feces, and KUGH 13, isolated from vomit. The EM1 and Ces3R/CESR2 primers had a specificity of 99.2% owing to false-positive results for KUGH 143, and the RE234 primer had a specificity of 98.3% owing to false-positive results for KUGH 143 and KUGH 13. Nakano *et al*. [23] reported that PCR results using the RE234 primer produced positive results for every emetic-toxin-producing *B. cereus* tested, yet a false positive for one diarrheal-toxin-producing *B. cereus* strain (ATCC 7004). Thus, the accuracy of each primer was as follows: 97.5% (CER), 95.6% (EM1), 96.3% (RE234), 89.4% (CES), and 83.1% (Ces3R/CESR2), indicating that the CER primer was more sensitive and accurate than all the other primers tested. Therefore, despite its low specificity, the CER primer can be used for the accurate screening of Korean emetic-toxin-producing *B. cereus* strains.

In conclusion, the results of this study revealed that the CER primer was useful for screening the emetic-toxin-producing *B. cereus* strains tested in this study. In addition, it is worth noting that the ability of PCR assays to identify and detect emetic-toxin-producing *B. cereus* should also be confirmed using other methods, such as a boar sperm assay, rat liver mitochondria assay, Hep-2 cell culture assay, and HPLC/MS analysis. However, further studies are still needed for the development of species-specific primers for the accurate detection of Korean emetic-toxin-producing *B. cereus* strains.

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