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Prevalence and Toxin Characteristics of *Bacillus thuringiensis* Isolated from Organic Vegetables

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The prevalence and toxin characteristics of *Bacillus thuringiensis* isolated from 39 organic vegetables were investigated. *B. thuringiensis* was detected in 30 out of the 39 organic vegetables (76.9%) with a mean value of 2.60 log CFU/g. Twenty-five out of the 30 *B. thuringiensis* isolates (83.3%) showed insecticidal toxicity against *Spodoptera exigua*. The *hblCDA*, *nheABC*, and *entFM* genes were found to be the major toxin genes, but the *ces* gene was not detected in any of the tested *B. thuringiensis* isolates. The hemolysin BL enterotoxin was detected in all 30 *B. thuringiensis* isolates (100%). The non-hemolytic enterotoxin complex was found in 27 out of 30 *B. thuringiensis* isolates (90.0%). The *B. thuringiensis* tested in this study had similar toxin gene characteristics to *B. cereus*, which possessed more than one toxin gene. *B. thuringiensis* could have the potential risk of foodborne illness based on the toxin genes and toxin-producing ability.

Keywords: *Bacillus thuringiensis*, enterotoxin, toxin gene, safety

Introduction

Bacillus cereus is a facultative anaerobic, gram-positive, endospore-forming pathogen accountable for diarrheal and emetic foodborne illness [1]. The diarrheal foodborne illness is caused by heat-labile toxins such as hemolysin BL (HBL), non-hemolytic enterotoxin (NHE), cytotoxin K (CytK), and enterotoxin FM (EntFM) [2]. The emetic illness is induced by a small, cyclic and acid-stable emetic toxin, [3]. *B. thuringiensis* produces crystal proteins (δ-endotoxin) encoded by *cry* genes, which present insecticidal activity [4]. *B. thuringiensis* is well known as a microbial insecticide that has been applied to reduce the amount of chemical pesticides [5]. *B. cereus* and *B. thuringiensis* are commonly isolated in soil, vegetables, and foods [5–7]. *B. cereus* and *B. thuringiensis* share a 16S rRNA gene sequence (similarity > 99%) [8]. They cannot be classified by genetic and phenotypic assays [9]. The distinguishing characteristic of *B. thuringiensis* is the presence of insecticidal crystal protein and *cry* genes [10].

The consumption of raw and minimally processed vegetables has increased in developed countries because fresh vegetables contain bioavailable nutrients such as vitamins, minerals, and phytochemicals [11, 12]. These vegetables are considered natural and healthy food, but concerns of residual chemical pesticides in fresh vegetables have been raised [13]. Thus, consumers have turned to organic vegetables (chemical pesticide-free vegetables). The Organization for Economic Cooperation and Development has predicted that biopesticides, including *B. thuringiensis* pesticide, may account for 20% in the world's pesticide market by 2020 to substitute for chemical pesticides [6, 14].

The total number of outbreaks of foodborne illness associated with fresh vegetables has been reported in developed countries [15–17]. *B. cereus* is a second risk priority group of foodborne illness in fresh agricultural products, and its contamination is one of the major problems in vegetables [18]. However, these previous studies could not discriminate between *B. cereus* and *B. thuringiensis*, and few cases of foodborne illness associated with *B. thuringiensis* have been reported [19, 20]. In the Korea Food Code, a *B. cereus* enumeration method was established, except *B. thuringiensis* cell counts. Thus, it is necessary to evaluate the pathogenic potential of *B. thuringiensis* isolated from organic vegetables. However, few studies have been performed on the prevalence of *B. thuringiensis* and its toxigenic properties.

Thus, this study was performed to determine the prevalence of *B. cereus* and *B. thuringiensis* in organic vegetables and to investigate the toxigenic potential based on the occurrence of toxin genes and production ability of enterotoxins as well as insecticidal activity.

Materials and Methods

Isolation, Identification, and Enumeration of *B. cereus*

Thirty-nine organic vegetables (26 leafy vegetables, 6 flowerhead brassicase, 4 fruiting vegetables, and 3 root and tuber vegetables) were purchased from retail markets in Gyeonggi-do, South Korea between May and July in 2014. Twenty-five grams of each sample was mixed with 225 ml of buffered peptone water (Oxoid Ltd, UK) in a sterile filter stomacher bag and homogenized by using a BagMixer stomacher (Interscience, France) for 2 min. One milliliter of homogenate was serially diluted (10-fold) in 0.85% saline. One milliliter (0.2 ml, 5 times) of each diluent was spread onto *B. cereus* rapid agar (BACARA; AES-Chemunex Inc., France) and then incubated at 30°C for 24 h. The BAKARA plate is a more suitable medium for *B. cereus* and *B. thuringiensis* than other selective media such as mannitol-egg yolk-polymyxin agar [21, 22]. The colonies that exhibited a pink-orange color on each BAKARA plate were counted, and one colony of each plate (total 5 colonies) was subcultured on 5% sheep blood agar (BA; Komed Life Science Co., Korea) at 30°C for 24 h. Biochemical identification of the selected colonies was conducted by using the Vitek-II system with the BCL card (bioMérieux, Inc., France) according to the manufacturer's directions.

Distinction of *B. thuringiensis* among *B. cereus* Strains

Microscopy observation was carried out to detect crystal proteins (δ-endotoxins) for the distinction of *B. thuringiensis* among *B. cereus* strains identified by the Vitek-II system. The simple staining procedure with TB carbol-fuchsin ZN (BD BBL Difco, USA) was used for the staining of cells after strains were cultured on tryptone soya agar (TSA; Oxoid) at 30°C for more than 96 h [23]. The crystal proteins were examined with an optical microscope (Axioskop 2 plus; ZEISS Inc., Germany). A PCR assay was conducted to detect the *cry* gene for confirmation of *B. thuringiensis* using a JinTech *B. cereus* PCR kit (Jinsung-UniTech Co., Korea) according to the manufacturer's instructions. *B. thuringiensis* KCTC 1509 was used as a reference strain.

Preparation of Bacterial Cultures for the Insecticidal Assay

To test the insecticidal activity, each *B. thuringiensis* strain was inoculated into nutrient broth (BD BBL Difco, USA) for overnight culture and then spread on five nutrient agar (KisanBio Co., Korea) plates and incubated at 30°C for 5 days. The occurrence of autolysis in the plates was examined with a phase contrast microscope (BX51; Olympus Co., Japan). Once it was confirmed, the bacterial lawns on the plates were harvested with sterile water and then collected using a centrifuge at 15,000 rpm at 4°C for 15 min. (Union 32R; Hanil Science Industrial Co., Korea). After centrifugation, the supernatants were discarded and the pellets were washed 3 times with sterile water and then finally resuspended with sterile water to make approximately 1.5×10^8 CFU/ml and stored at 4°C before further experiments. *B. cereus* ATCC 14579 and *B. thuringiensis* ATCC 10792 were used as reference strains.

Insecticidal Activity of *B. thuringiensis* Strains

Tests of the insecticidal activity against *Plutella xylostella* were performed according to a leaf dip bioassay with minor modification, using cabbage leaf [24]. Leaves (3×3 cm^2) were dipped into 20 ml of the resuspended pellets of *B. thuringiensis* and then allowed to be dry at room temperature. Three-fifths stage *P. xylostella* larvae were transferred onto the five plates. Dead or live larvae were counted every 24 h for 72 h to calculate the lethality rate (%). Tests of insecticidal activity against *Spodoptera exigua* and *Spodoptera litura* were performed according to Jin *et al*. [25]. The prepared pellets comprising *B. thuringiensis* were added to artificial food (1 g) on the plates. Three-fifths stage *S. exigua* and *S. litura* larvae were transferred onto the five plates. The instar of the larvae was confirmed by exuvium. Dead and live larvae were counted every 24 h for 120 h, to calculate the lethality rate (%). The experiments were replicated three times.

Detection of Enterotoxin and Emetic Toxin Genes

The strains identified as *B. cereus* or *B. thuringiensis* from each sample were streaked on TSA (Oxoid) and incubated at 30°C for 24 h. The DNA templates were extracted using the boiling method for the PCR assay according to the previously described protocol [26]. PCR amplification with a 20 µl reaction mixture consisting of AccuPower PCR PreMix (Bioneer Co., Korea), 50 ng of DNA template, and 500 nmol/l of each primer was conducted using a thermal cycler (Mastercycler Gradient S; Eppendorf AG, Germany). The PCR conditions and primer pair sequences used for amplifying the *hblCDA*, *nheABC*, *entFM*, *cytK*, and *ces* genes have been previously reported by Ngamwongsatit *et al*. [27], Yang *et al*. [28], Ghelardi *et*

al. [29], and Lee *et al*. [16], respectively. The PCR assay was 95°C for 30 sec, followed by 35 cycles of 95°C for 30 sec, 60°C for 60 sec, and 72°C for 60 sec, and a final extension at 72°C for 5 min. The primer sequences and product sizes are as follows; *hblC* (F: CCTATCAATACTCTCGCAA; R: TTTCCTTTGTTATACGCTGC; 695 bp), *hblD* (F: GAAACAGGGTCTCATATTCT; R: CTGCATCTT TATGAATATCA; 1,018 bp), *hblA* (F: GCAAAATCTATGAATGCCTA; R: GCATCTGTTCGTAATGTTTT; 884 bp), *nheA* (F: ATTACAGGG TTATTGGTTACAGCAGT; R: AATCTTGCTCCATACTCTCTT GGATGCT; 475 bp), *nheB* (F: GTGCAGCAGCTGTAGGCGGT; R: ATGTTTTTCCAGCTATCTTTCGCAAT; 328 bp), *nheC* (F: GCG GATATTGTAAAGAATCAAAATGAGGT; R: TTTCCAGCTATC TTTCGCTGTATGTAAAT; 557 bp), *entFM* (F: AAAGAAATTAAT GGACAAACTCAAACTCA; R: GTATGTAGCTGGGCCTGTACGT; 596 bp), *cytK* (F: ATCGGKCAAAATGCAAAAACACAT; R: ACC CAGTTWSCAGTTCCGAATGT; 800 bp), and *ces* (F: TTGTTG GAATTGTCGCAGAG; R: GTAAGCGAACCTGTCTGTAACAACA; 405 bp). The amplified products were separated by electrophoresis on 2% agarose gel and observed using a UV transilluminator (Gel Doc 2000; Bio-Rad Inc., USA). *B. cereus* ATCC 14579 and F4810/72 were used as reference strains.

Detection of Enterotoxins and Emetic Toxin

To detect enterotoxins such as HBL enterotoxin and NHE, all strains were cultured in tryptone soya broth (TSB; Oxoid) at 30°C for 24 h. The HBL enterotoxin was detected using the enterotoxin-reversed passive latex agglutination (BCET-RPLA) kit (Oxoid), and NHE was visualized with the *Bacillus* diarrheal enterotoxin visual immunoassay (BDE-VIA) kit (3M Tecra Diagnostics Pty LTD, UK). Emetic toxin was detected using Singlepath Emetic Tox Mrk (Merck, Germany), which is a confirmation test kit for the detection of co-expressed marker protein produced with emetic toxin, simultaneously. All strains of *B. cereus* and *B. thuringiensis* were inoculated into 1% glucose-casein hydrolysate glucose yeast extract broth (CGY; Merck) and incubated at 30°C for 24 h. One milliliter of each culture was centrifuged for 5 min at 10,000 ×*g* and the supernatants were examined for detection of emetic toxin using the immune-chromatographic rapid test according to the manufacturer's directions. *B. cereus* ATCC 14579 and F4810/72 were used as reference strains.

Results and Discussion

Prevalence of *B. cereus* and *B. thuringiensis* in Organic Vegetables

To estimate the prevalence of *B. cereus* and *B. thuringiensis* in organic vegetables, their biochemical identification was conducted using the BAKARA plate and Vitek-II system (Table 1). *B. cereus* and *B. thuringiensis* are detected on BAKARA plate but not distinguished in this plate. *B. cereus* including *B. thuringiensis* was detected in 35 out of 39 (89.7%) organic vegetables and their average number was 2.79 log CFU/g. Kang *et al*. [30] reported that *B. cereus* was detected in 16 out of 30 salads (53.3%) and the mean value was 1.62 log CFU/g. The contamination level of *B. cereus* in lettuce ranged from 1.23 to 3.25 log CFU/g [31] and the contamination level of *B. cereus* in organic vegetables ranged from 1.2 to 4.0 log CFU/g [32]. The prevalence of *B. cereus* in Sunsik ingredients was 48.1% [33]. The detection rate of *B. cereus* and *B. thuringiensis* (89.7%) in this study was higher than that of previous studies.

Table 1. Prevalence of *Bacillus cereus* in organic vegetables.

Sources	No. of detection samples (%)	Mean ± SD[a,b]
Leafy vegetables (*n* = 26)	25 (96.2)	3.51 ± 1.24
Flowerhead brassicase (*n* = 6)	4 (66.7)	1.47 ± 1.2
Fruiting vegetables (*n* = 4)	3 (75.0)	1.52 ± 1.69
Root and tuber vegetables (*n* = 3)	3 (100)	0.81 ± 0.25
Total (*n* = 39)	35 (89.7)	2.79 ± 1.59

[a]SD: standard deviation.

[b]Unit: log CFU/g.

The distinguishing characteristic of *B. thuringiensis* against *B. cereus* is the presence of insecticidal crystal proteins (δ-endotoxin) that are encoded by *cry* genes [10]. Five colonies counted as *B. cereus* on BAKARA plate from each sample were investigated to classify *B. thuringiensis*. The number of *B. thuringiensis* was calculated by multiplying the number of *B. cereus* by the detection ratio of *B. thuringiensis* among five colonies. The microscope photographs of crystal proteins (δ-endotoxin) are presented in Fig. 1. *B. thuringiensis* was

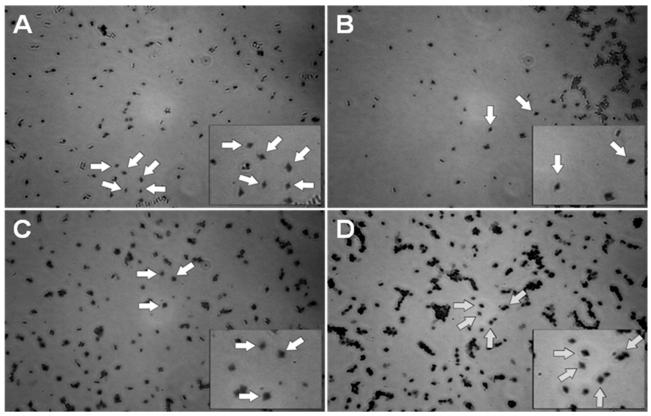

Fig. 1. Microscope photographs showing crystal proteins (δ-endotoxin) of *B. thuringiensis* KCTC 1509 reference strain (**A**), and *B. thuringiensis* isolated from leafy vegetables (**B**), flowerhead brassicase (**C**), and fruiting vegetables (**D**).

Table 2. Prevalence of *B. thuringiensis* in organic vegetables.

Isolates	No. of samples containing *B. thuringiensis* (%)	Mean ± SD[a,b]
Leafy vegetables (*n* = 26)	23 (88.5)	3.09 ± 1.47
Flowerhead brassicase (*n* = 6)	4 (66.7)	2.02 ± 0.23
Fruiting vegetables (*n* = 4)	3 (75.0)	1.89 ± 1.66
Root and tuber vegetables (*n* = 3)	0 (0.0)	-
Total (*n* = 39)	30 (76.9)	2.60 ± 1.59

[a]STD: standard deviation.

[b]Unit: log CFU/g.

detected in 30 out of 39 organic vegetables (76.9%) with a mean value of 2.60 log CFU/g (Table 2). The highest frequency was observed in leafy vegetables (23 out of the 26 samples, 88.5%). Kim *et al*. [34] reported that *B. cereus* and *B. thuringiensis* spores were isolated from 148 out of 189 (78.3%) and 13 out of 189 (6.9%) rice samples. Thirty-one strains of *B. thuringiensis* were classified as 40 *B. cereus*-like strains isolated from ready-to-eat food in a Danish retail market [7]. Although we investigated a small number of samples, most organic vegetables were contaminated with *B. thuringiensis*.

Insecticidal Activity of *B. thuringiensis* Strains

To evaluate the insecticidal activity of *B. thuringiensis* isolates, insecticidal assays were performed with Lepidoptera larvae. Twenty-five out of 30 *B. thuringiensis* isolates (83.3%) tested in this study showed insecticidal toxicity against Lepidoptera larvae. The *B. thuringiensis* 1411, 1417, 1424, and 1429 strains isolated from leaf vegetables and *B. thuringiensis* 1414 strain isolated from fruiting vegetables did not present insecticidal toxicity (Table 3). *B. thuringiensis* ATCC 10792 strain showed insecticidal toxicity against *Plutella xylostella* and *Spodoptera litura*, but showed non-insecticidal toxicity against *Spodoptera exigua*. These results are in accordance with findings of a previous report, which showed that some *B. thuringiensis* isolates possessing the *cry* gene and crystal protein showed non-insecticidal toxicity [35].

Possession of Enterotoxin and Emetic Toxin Genes

The distributions of *hblC*, *hblD*, *hblA*, *nheA*, *nheB*, *nheC*, *entFM*, *cytK*, and *ces* genes among *B. cereus* and *B. thuringiensis* strains isolated from organic vegetables are presented in Table 4. The percentage of presence of these genes in *B. cereus* isolates was 100%, except for the *cytK* (0.0%) and *ces* gene (0.0%). The percentage of occurrence of these toxin genes in *B. thuringiensis* isolates without insecticidal activity was 100.0%, 80.0%, 80.0%, 100.0%, 100.0%, 100.0%, 100.0%, 20.0%, and 0.0%, respectively. The percentage of

Table 3. Insecticidal toxicity of *B. thuringiensis* against Lepidoptera larvae.

B. thuringiensis strains	Microscope (crystal toxin)	Insecticidal toxicity		
		Plutella xylostella	*Spodoptera litura*	*Spodoptera exigua*
1401	+	+	+	+
1402	+	+	+	+
1408	+	+	+	+
1411	+	-	-	-
1412	+	+	+	+
1413	+	+	+	+
1414	+	-	-	-
1415	+	+	+	+
1416	+	+	+	+
1417	+	-	-	-
1418	+	+	+	+
1419	+	+	+	+
1420	+	+	+	+
1421	+	+	+	+
1422	+	+	+	+
1423	+	+	+	+
1424	+	-	-	-
1425	+	+	+	+
1426	+	+	+	+
1429	+	-	-	-
1430	+	+	+	+
1431	+	+	+	-
1432	+	+	+	+
1433	+	+	+	+
1434	+	+	+	+
1435	+	+	+	+
1436	+	+	+	+
1437	+	+	+	+
1438	+	+	+	+
1439	+	+	+	+
B. cereus ATCC 14579		-	-	-
B. thuringiensis ATCC 10792		+	+	-

Table 4. Toxin gene profiles and toxin production ability of *B. cereus* isolated from organic vegetables.

Source		Isolates	Toxin gene									Toxin production		
			hblC	*hblD*	*hblA*	*nheA*	*nheB*	*nheC*	*entFM*	*cytK*	*ces*	HBL[a]	NHE[b]	Cereulide[c]
B. cereus	Leafy vegetables	1427	+	+	+	+	+	+	+	-	-	+	+	-
		1428	+	+	+	+	+	+	+	-	-	+	+	-
	Roots and tuber vegetables	1404	+	+	+	+	+	+	+	-	-	+	+	-
		1406	+	+	+	+	+	+	+	-	-	+	+	-
		1409	+	+	+	+	+	+	+	-	-	+	+	-
	Total (n = 5)		5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	0 (0.0)	0 (0.0)	5 (100)	5 (100.0)	0 (0.0)

[a]The *B. cereus* enterotoxin reversed passive latex agglutination (BCET-RPLA) was used to detect hemolysin BL (HBL) enterotoxin.

[b]The *Bacillus* diarrheal enterotoxin visual immunoassay (BDE-VIA) kit was used to detect non-hemolytic enterotoxin (NHE) of *B. cereus* and *B. thuringiensis*.

[c]The Singlepath Emetic Tox Mrk kit was used to detect co-expressed marker protein produced with emetic toxin, simultaneously.

occurrence of these toxin genes in *B. thuringiensis* isolates with insecticidal activity was 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 36.0%, and 0.0%, respectively. These results indicated that whereas *hblCDA*, *nheABC*, and *entFM* were found to be the major toxin genes, the *ces* gene was not detected in any of the tested *B. cereus* and *B. thuringiensis* isolates.

These results are in accordance with findings of previous studies in which *hblCDA* genes were detected in 90.0% of *B. cereus* isolated from clinical and food samples [36], and in 86% of Sunsik samples in Korea [37]. The rate of occurrence of *hbl* genes among food-isolated *B. cereus* was 84% in Belgium [38]. The *nheA*, *nheB*, and *nheC* genes were detected in 74–97% isolates from Sunsik [37] and in 97.8%, 92.2%, and 90.9% of clinical, grain, and food strains in Korea, respectively [36]. The EntFM enterotoxin encoded in *entFM* gene does not have hemolytic activity. EntFM has been indicated to be cytotoxic to Vero cells [39]. The toxin is thought to be a *B. cereus* cell wall peptidase related with adhesion, biofilm formation, and virulence [40]. Previous studies reported that all *B. cereus* isolates from Sunsik carried the *entFM* gene [37] and all of nine Antarctic *B. thuringiensis* isolates carried the *entFM* gene [41]. CytK enterotoxin was implicated in a severe foodborne illness, and hemolytic and cytotoxic disease, related with three deaths in France [42, 43]. Distribution of the *cytK* gene in *B. cereus* varied from 13% to 57% [44, 36, 43]. The *cytK* gene was detected in 5 out of 13 (38.5%) and 1 out of 21 (4.8%) *B. thuringiensis* strains from rice products [46], milk, and soil [47]. It has been reported that the *ces* gene was found in only 0.05% of *B. cereus* strains isolated from a dairy production chain [48]. In the present study, the detection ratios of *entFM*, *cytK*, and *ces* genes in *B. cereus* and *B. thuringiensis* were similar. Considering the prevalence of toxin genes characterized in this study, *B. thuringiensis* might be a potential food safety risk in spite of the wide use of insecticidal pesticide in organic agriculture. Thus, we investigated whether these *B. thuringiensis* isolates possessing enterotoxin genes could produce the toxins.

Enterotoxins and Emetic Toxin Production Ability

The major enterotoxins associated with diarrheal disease caused by *B. cereus* are HBL and NHE among HBL, NHE, CytK, and EntFM [49, 27]. The HBL enterotoxin comprises binding protein B and two lytic subunits (L1 and L2) encoded by *hblC*, *hblA*, and *hblD* [50]. The NHE complex consists of NheA, NheB, and NheC components encoded by *nheA*, *nheB*, and *nheC* genes, respectively [51]. The HBL and NHE complexes are expressed only when all three genes are present [52]. The emetic illness is caused by a small, and acid-stable emetic toxin produced by *B. cereus* [53]. The ability to produce two enterotoxins and the emetic toxin was assessed to estimate the potential risk of foodborne illness caused by *B. cereus* and *B. thuringiensis* strains, using the BCET-RPLA, BDE-VIA, and Singlepath Emetic Tox Mrk kits (Tables 4 and 5). The emetic toxin was not detected in any of the *B. cereus* and *B. thuringiensis* isolates, whereas the HBL enterotoxin was detected in all of five *B. cereus* (100.0%) and 30 (100.0%) *B. thuringiensis* isolates. The NHE complex was found in all isolates of *B. cereus* (100.0%) and all of *B. thuringiensis* isolates (100.0%) without insecticidal activity, but 22 out of 25 (88.0%) *B. thuringiensis* isolates with insecticidal activity produced NHE toxin.

These results are in agreement with the previous findings, which showed that HBL production was detected in 24 out of 28 *B. thuringiensis* isolates (85.7%) and all of 59 *B. thuringiensis* isolates (100.0%) [7, 54]. HBL enterotoxin was produced in 80.0%, 85.9%, and 81.8% of *B. cereus* isolates from the clinical, grain, and food samples, respectively [36]. NHE positive rates were found in 4 out of 13 *B. cereus* isolates (30.7%) and

Table 5. Toxin gene profiles and toxin production ability of *B. thuringiensis* isolated from organic vegetables.

Source		Isolates	Toxin gene									Toxin production		
			hblC	*hblD*	*hblA*	*nheA*	*nheB*	*nheC*	*entFM*	*cytK*	*ces*	HBL[a]	NHE[b]	Cereulide[c]
B. thuringiensis (non-insecticidal activity)	Leafy vegetables	1411	+	+	+	+	+	+	+	-	-	+	+	-
		1417	+	+	-	+	+	+	+	-	-	+	+	-
		1424	+	+	+	+	+	+	+	-	-	+	+	-
		1429	+	+	+	+	+	+	+	+	-	+	+	-
	Fruiting vegetables	1414	+	-	+	+	+	+	+	-	-	+	+	-
	Subtotal (n = 5)		5 (100)	4 (80)	4 (80)	5 (100)	5 (100)	5 (100)	5 (100)	1 (20)	0 (0.0)	5 (100)	5 (100.0)	0 (0.0)
B. thuringiensis (insecticidal activity)	Leafy vegetables	1401	+	+	+	+	+	+	+	-	-	+	+	-
		1408	+	+	+	+	+	+	+	-	-	+	+	-
		1415	+	+	+	+	+	+	+	-	-	+	-	-
		1416	+	+	+	+	+	+	+	-	-	+	+	-
		1418	+	+	+	+	+	+	+	-	-	+	+	-
		1419	+	+	+	+	+	+	+	-	-	+	+	-
		1420	+	+	+	+	+	+	+	+	-	+	+	-
		1421	+	+	+	+	+	+	+	-	-	+	+	-
		1422	+	+	+	+	+	+	+	-	-	+	+	-
		1423	+	+	+	+	+	+	+	-	-	+	+	-
		1425	+	+	+	+	+	+	+	-	-	+	+	-
		1426	+	+	+	+	+	+	+	-	-	+	+	-
		1430	+	+	+	+	+	+	+	+	-	+	+	-
		1431	+	+	+	+	+	+	+	+	-	+	+	-
		1432	+	+	+	+	+	+	+	+	-	+	+	-
		1433	+	+	+	+	+	+	+	-	-	+	-	-
		1434	+	+	+	+	+	+	+	-	-	+	+	-
		1435	+	+	+	+	+	+	+	+	-	+	+	-
		1436	+	+	+	+	+	+	+	+	-	+	+	-
	Flowerhead brassicase	1402	+	+	+	+	+	+	+	-	-	+	+	-
		1437	+	+	+	+	+	+	+	+	-	+	+	-
		1438	+	+	+	+	+	+	+	+	-	+	+	-
		1439	+	+	+	+	+	+	+	+	-	+	-	-
	Fruiting vegetables	1412	+	+	+	+	+	+	+	-	-	+	+	-
		1413	+	+	+	+	+	+	+	-	-	+	+	-
	Subtotal (n = 25)		25 (100)	25 (100)	25 (100)	25 (100)	25 (100)	25 (100)	25 (100)	9 (36.0)	0 (0.0)	25 (100)	22 (88.0)	0 (0.0)
Total (n = 30)			30 (100)	29 (96.7)	29 (96.7)	30 (100)	30 (100)	30 (100)	30 (100)	10 (33.3)	0 (0.0)	30 (100)	27 (90.0)	0 (0.0)

[a]The *B. cereus* enterotoxin reversed passive latex agglutination (BCET-RPLA) was used to detect hemolysin BL (HBL) enterotoxin.

[b]The *Bacillus* diarrheal enterotoxin visual immunoassay (BDE-VIA) kit was used to detect non-hemolytic enterotoxin (NHE) of *B. cereus* and *B. thuringiensis*.

[c]The Singlepath Emetic Tox Mrk kit was used to detect co-expressed marker protein produced with emetic toxin, simultaneously.

15 out of 41 (36.6%) *B. thuringiensis* isolates [44, 55]. Emetic *B. cereus* strains possessing the *ces* gene encoding emetic toxin are rare [56, 57]. The toxin production characteristics of *B. thuringiensis* tested in this study were similar to those of *B. cereus*. Four cases of outbreaks associated with *B. thuringiensis* in Canada have been reported [19], while Janes *et al*. [58] reported that *B. thuringiensis* produced HBL and NHE enterotoxins but did not cause gastrointestinal illness. Considering organic vegetables are commonly consumed without further mild heat treatment, *B. thuringiensis* might be a potential hazard that causes foodborne illness.

In conclusion, the toxin production characteristics of *B. thuringiensis* isolates tested in this study were similar to those of *B. cereus*. These results suggest that *B. thuringiensis* might have potential risk of foodborne illness based on its toxin genes and toxin-producing ability.

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