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Combination of Enrichment and PCR in Rapid Semi-Quantification of *Bacillus cereus* in Fresh-Cut Vegetables

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ABSTRACT - Widespread consumption of fresh-cut vegetables without cooking results in ingestion of major foodborne pathogens including *Bacillus cereus.* In this study, we aimed to develop a method to rapidly detect *B. cereus* in fresh-cut vegetables by combining commercial PCR analysis with enrichment of the pathogenic levels. A mixture of *B. cereus* strains (KCTC1013, KCTC1014, KCTC1092, KCTC1094, and KCTC3624) was inoculated on the surface of fresh-cut cabbage lettuce (20 g) and baby leafy vegetables (10 g) to concentration 1, 2, 3, 4, and 5 log CFU/g. Eighty milliliters of TSB with 0.15% polymyxin B was used for cabbage lettuce, and 90 mL of medium was used for baby leafy vegetables and incubated at 42°C for 0, 2, 3, 4, 5, 6, and 7 h. One milliliter of the enriched media was plated on mannitol-egg yolk-polymyxin agar for quantification, and another 1 mL was used for DNA extraction for PCR analysis. Additionally, the minimum number of sub-samples to be tested from a pack of fresh-cut vegetable samples was determined using 5 sub-samples. The results from this study showed that for detecting *B. cereus* in fresh-cut cabbage lettuce, 3, 4, 5, 6, and 7 h enrichment were required to at least detect 5, 4, 3, 2, and 1 log CFU/g of *B. cereus*, respectively. *B. cereus* in fresh-cut baby leafy vegetables could be detected after 2, 3, 4, 5, and 6 h of enrichment at 5, 4, 3, 2, and 1 log CFU/g, respectively, using a combination of enrichment and PCR analysis. To determine if a pack of fresh-cut vegetable is positive, the minimum number of sub-samples should be 3. These results can be used to develop a rapid detection method to semi-quantify *B. cereus* in fresh-cut vegetable samples combining enrichment and PCR.

Key words : *Bacillus cereus*, Fresh-cut vegetables, Rapid detection, Enrichment, PCR

Ready-to-use products are produced because of increasing demand for minimally processed and fresh-cut vegetables. Fresh-cut products are vegetables or fruits that were peeled and/or trimmed and/or cut into usable products according to the International Fresh-cut Produce Association (IFPA)[2]. In particular, fresh-cut vegetables are generally consumed without cooking, and thus, the safety of these foods in the context of foodborne pathogens is critical.

Bacillus cereus is a pathogenic bacterium typically present in plants[1,3] and can therefore be detected in raw vegetable seed sprouts, mainly in salads[3]. Valero et al.[1] showed that 8.3% of packaged and refrigerated American salads were contaminated with *B. cereus*. Becker et al.[4] also detected more than 3 log CFU/g of *B. cereus* sensu lato (*B. cereus*, *Bacillus thuringiensis*, and *Bacillus anthracis*) in 16.7% vegetable-based salads. Kim et al.[5] and Chon et al.[6] detected *B. cereus* in 27.3-30.8% of fresh-cut vegetables, and 48% of retail vegetable salads and sprouts in S. Korea. *B. cereus* is therefore considered to be a highly prevalent pathogen in vegetables, especially the ones consumed raw. These products undergo no or minimal decontamination, such as washing with water, and also have a short shelf life. Hence, it is necessary to develop a method that can rapidly detect *B. cereus* in these products before they leave the processing facility.

According to the Bacteriological Analytical Manual (BAM), *B. cereus* detection in foods should be conducted through biochemical testing on the suspected colonies that are pink in color and are surrounded by the precipitate zone on mannitol-egg yolk-polymyxin (MYP) agar[7]. These methods, although useful in precisely confirming the presence of *B. cereus*, are time-consuming. Thus, conventional PCR, real-time PCR, multiplex PCR, enzyme-linked immunosorbent assay (ELISA), and DNA microarray can be used as alternative methodologies for the identification of *B. cereus* in suspicious colonies[8]. These rapid and accurate methods are typically designed for the

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identification of single colonies, but they cannot be applied directly to the samples for quantification. Additionally, the detection limits for these methods are relatively high; therefore, the sample has to be enriched prior to these methods being used. However, quantification upon enrichment is challenging. Therefore, it is necessary to develop a method of detecting *B. cereus* that combines the accuracy of the PCR method and the benefits of enrichment. Choi et al.[9] proposed such a method for the detection of *Escherichia coli*, but this method could not be used to quantify *E. coli*.

Therefore, the objective of this study was to develop a method combining enrichment and PCR, for the rapid detection of *B. cereus* in food samples.

Materials and Methods

Inocula preparation

A mixture of *B. cereus* strains KCTC1013, KCTC1014, KCTC1092, KCTC1094, and KCTC3624 was used in this study. A colony of each strain was cultured in 10 mL tryptic soy broth (TSB, Becton, Dickinson and Company, Sparks, MD, USA) at 30°C for 24 h. Aliquots of 0.1 mL of the cultures were sub-cultured in 10 mL of fresh TSB at 30°C for 24 h. Subcultures of the different strains were then mixed and centrifuged at 1,912×g at 4°C for 15 min. The pellet was washed twice with 50 mL phosphate buffered saline (PBS, 0.2 g of KH_2PO_4, 1.5 g of $Na_2HPO_4 \cdot 7H_2O$, 8.0 g of NaCl, and 0.2 g of KCl in 1 L of distilled water, pH 7.4) and subsequently resuspended and diluted with PBS to prepare 3-, 4-, and 5-log CFU/mL inocula.

Sample preparation and inoculation

Fresh-cut vegetables (cabbage lettuce and baby leafy vegetables) were purchased from a market in Seoul, S. Korea. Portions of 20 g and 10 g cabbage lettuce and baby leafy vegetables, respectively, were placed in filter bags (3M, St. Paul, MN, USA). The inocula (0.1 mL) were used to obtain fresh-cut vegetable samples with *B. cereus* at concentrations of 1-, 2-, 3-, 4-, and 5-log CFU/g, and the samples were massaged 20 times by hand. Samples were then left under laminar flow for 15 min to allow *B. cereus* attachment. Eighty milliliters and 90 mL TSB, each supplemented with 0.15% polymyxin B (Oxoid, Basingstoke, Hampshire, UK), were used for cabbage lettuce and baby leafy vegetables, respectively, and placed in filter bags.

Enrichment and quantification of *B. cereus*

Enrichment of *B. cereus* in the fresh-cut vegetable samples was performed by incubating the samples with 1-, 2-, 3-, 4-, and 5-log CFU/g of *B. cereus* at 42°C for 0, 2, 3, 4, 5, 6, and 7 h. One milliliter of the enriched media for each sample corresponding to a particular time-point was serially diluted with 0.1% buffered peptone water (BPW; Becton, Dickinson and Company), and plated on MYP (Becton, Dickinson and Company) agar. The plates were incubated at 30°C for 24 h, and the characteristic pink-red colonies were manually counted.

DNA extraction

The 1 mL aliquots of the enriched samples were centrifuged at 14,000 rpm at 4°C for 5 min, and the pellet washed with 30 μL of distilled water. The suspensions were boiled at 100°C for 10 min and left at room temperature for 2 min. The supernatants were used as DNA templates after centrifugation at 14,000 rpm at 4°C for 3 min.

B. cereus multiplex PCR

A multiplex PCR kit (Jinsung-UniTech Co., Goyang, Korea) was used to detect *B. cereus* in the enriched samples. This kit was used to detect *cry1* (138 bp), *groEL* (250 bp), and *ces* (405 bp) genes using specific primers. *cry1* was detected only in *B. thuringiensis*, *groEL* was detected in both *B. cereus* and *B. thuringiensis*, and *ces* was detected only in emetic toxin-producing *B. cereus*[10]. The detection limit for the *B. cereus* and *B. thuringiensis* multiplex PCR kit (Jinsung-UniTech Co.) was 5 log CFU/mL[11].

Determination of the number of sub-samples

Several sub-samples from the package had to be examined to determine if a pack of fresh-cut vegetable samples was contaminated with *B. cereus*. Hence, the number of sub-samples was determined. Fresh-cut cabbage lettuce and baby leafy vegetables were dipped into *B. cereus* inocula for 2 min to obtain 3 log CFU/g, and the samples were drained for 15 min. The fresh-cut vegetable samples were placed in sample bags (3M). Five sub-samples of fresh-cut cabbage lettuce (20 g) and baby leafy vegetables (10 g) were withdrawn from a sample bag and placed in filter bags. TSB supplemented with 0.15% polymyxin B (90 mL) (Oxoid, UK) was placed into the sub-samples and incubated at 42°C for 24 h. The bacterial cell counts were quantified by plating samples on MYP (Becton, Dickinson and Company) agar as described previously. DNA was extracted at 0, 2, 3, 4, 5, 6, and 7 h, and used for PCR analysis using the methods described above.

Results and Discussion

According to Lee et al.[10], the *B. cereus* detection limit of the *B. cereus* multiplex PCR kit (Jinsung-UniTech Co.) was

Table 1. Results (the number of positive samples/the number of total samples) of polymerase chain reaction analysis by *Bacillus cereus* multiplex PCR kit and *B. cereus* cell counts (n=5; mean ± standard deviation, log CFU/g) after enrichment of *B. cereus* in the fresh-cut cabbage lettuces at 42°C for 0 h, 2 h, 3 h, 4 h, 5 h, 6 h, and 7 h

Enrichment time (h)	Initial concentration of *Bacillus cereus* (log CFU/g)									
	1		2		3		4		5	
	PCR results	Cell counts	PCR results	Cell counts	PCR results	Cell counts	PCR results	Cell counts	PCR results	Cell counts
0	0/5	0.9 ± 0.4	0/5	1.9 ± 0.3	0/5	2.9 ± 0.2	0/5	3.6 ± 0.2	2/5	4.6 ± 0.2
2	0/5	1.8 ± 0.5	0/5	2.7 ± 0.1	0/5	3.3 ± 0.5	0/5	4.7 ± 0.2	2/5	5.8 ± 0.3
3	0/5	3.0 ± 0.3	0/5	5.0 ± 0.2	0/5	5.1 ± 0.2	0/5	6.0 ± 0.2	5/5	7.4 ± 0.2
4	0/5	4.2 ± 0.4	0/5	5.3 ± 0.3	0/5	6.2 ± 0.4	4/5	7.3 ± 0.3	5/5	8.3 ± 0.3
5	0/5	5.4 ± 0.4	0/5	6.4 ± 0.5	2/5	7.0 ± 0.2	4/5	7.7 ± 0.9	4/5	8.6 ± 0.3
6	1/5	6.1 ± 0.7	4/5	6.7 ± 0.7	4/5	7.8 ± 0.6	4/5	8.6 ± 0.3	4/5	8.6 ± 0.0
7	3/5	7.3 ± 0.6	3/5	7.7 ± 0.3	3/5	8.5 ± 0.3	-†	-	-	-

†Not analyzed.

approximately 5 log CFU/mL, and the enrichment temperature was determined to be 42ºC for detecting *B. cereus* at 30ºC, 37ºC, and 42ºC. Hence, *B. cereus* in fresh-cut cabbage lettuces inoculated at 1, 2, 3, 4, and 5-log CFU/g was enriched at 42ºC for 0 h, 2 h, 3 h, 4 h, 5 h, 6 h, and 7 h. Among the 5 samples in each enrichment time, the 1 log CFU/g inoculated samples became positive after 6 h of enrichment, and 3 samples became positive after 7 h of enrichment (Table 1). The cell counts of *B. cereus* in the fresh-cut cabbage lettuce samples after enrichment were 6.1 (6 h) and 7.3 log CFU/g (7 h). The fresh-cut cabbage lettuce samples inoculated with a *B. cereus* concentration of 2 log CFU/g became positive in 4 samples after 6 h enrichment and 3 out of 5 samples tested positive after 7 h-enrichment. The cell counts of *B. cereus* after enrichment were 6.7 (6 h) and 7.7 log CFU/g (7 h). The samples inoculated with a *B. cereus* concentration of 3 log CFU/g became positive in 2, 4, and 3 out of 5 samples after 5 h-, 6 h-, and 7 h-enrichment, respectively. The cell counts of *B. cereus* after enrichment were 7.0 (5 h), 7.8 (6 h), and 8.5 log CFU/g (7 h). Four of the 5 samples inoculated with a *B. cereus* concentration of 4-log CFU/g became positive after 4 h-, 5 h-, and 6 h-enrichment. The cell counts of *B. cereus* after enrichment were 7.3 (4 h), 7.7 (5 h), and 8.6 log CFU/g (6 h). In the case of samples inoculated with a *B. cereus* concentration of 5 log CFU/g, 2 out of 5 samples became positive after 0 h- and 2 h-enrichment, all samples became positive after 3 h- and 4 h-enrichment, and 4 samples became positive after 5 h- and 6 h-enrichment. The cell counts of *B. cereus* after enrichment were 4.6 (0 h), 5.8 (2 h), 7.4 (3 h), 8.3 (4 h), 8.6 (5 h), and 8.6 log CFU/g (7 h) (Table 1). These results indicate that 3 h-, 4 h-, 5 h-, 6 h-, and 7 h-enrichment time is required to detect 5, 4, 3, 2, and 1 log CFU/g of *B. cereus* in fresh-cut cabbage lettuce samples.

In the case of the fresh-cut baby leafy vegetables, out of the 1 log CFU/g of *B. cereus* inoculated samples, 1 sample became positive after 5 h enrichment, and 3 and 4 samples became positive after 6 h- and 7 h-enrichment respectively (Table 2). The *B. cereus* cell count in fresh-cut baby leafy vegetable samples after enrichment were 5.3 (5 h), 6.1 (6 h), and 7.3 log CFU/g (7 h). Only one sample inoculated with a *B. cereus* concentration of 2 log CFU/g became after 3-4 h-enrichment, and 4, 4, and 3 samples became positive after 5 h-, 6 h-, and 7 h-enrichment, respectively. The *B. cereus* cell count after enrichment was 4.7 (3 h), 5.3 (4 h), 6.2 (5 h), 7.0 (6 h), and 7.9 log CFU/g (7 h). The 3-log CFU/g inoculated samples became positive in 2 samples after 3 h-enrichment, and 4 of 5 samples became positive after 4 h-, 5 h-, 6 h-, and 7 h-enrichment. The *B. cereus* cell count after enrichment was 5.8 (3 h), 7.0 (4 h), 6.6 (5 h), 7.3 (6 h), and 8.5 log CFU/g (7 h), respectively. Out of the 4-log CFU/g inoculated samples, *B. cereus* was detected in 4, 4, 4, and 3 samples out of 5 samples after 3 h-, 4 h-, 5 h-, and 6 h-enrichment, respectively. *B. cereus* cell counts after enrichment were 6.7 (3 h), 7.9 (4 h), 7.6 (5 h), and 7.8 log CFU/g (6 h). In the samples inoculated with 5 log CFU/g, 2 samples were positive at 0 h enrichment and 4 of 5 samples were positive after 2 h-, 3 h-, and 4 h-enrichment. The *B. cereus* cell counts after enrichment were 5.4 (0 h), 6.1 (2 h), 7.6 (3 h), and 8.8. log CFU/g (4 h) (Table 2). These results showed that lower inoculation levels needed longer enrichment times to be detected as positive by PCR. This implies that knowledge of the enrichment time required for positive detection would allow estimation of *B. cereus* contamination levels.

Although the detection limit of *B. cereus* with the

Table 2. Results (the number of positive samples/the number of total samples) of polymerase chain reaction analysis by *Bacillus cereus* multiplex PCR kit and *B. cereus* cell counts (n=5; mean ± standard deviation, log CFU/g) after enrichment of *B. cereus* in the fresh-cut baby leafy vegetables at 42°C for 0 h, 2 h, 3 h, 4 h, 5 h, 6 h, and 7 h

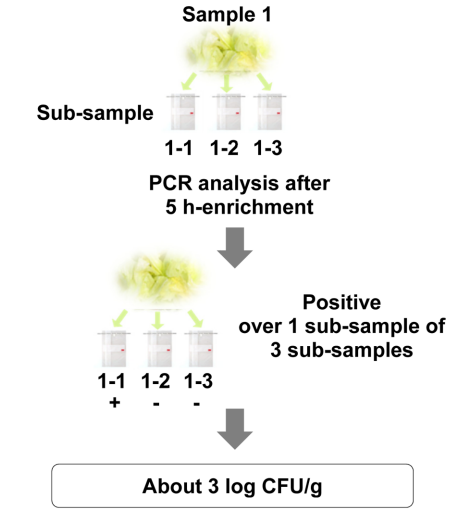
Enrichment time (h)	Initial concentration of *Bacillus cereus* (log CFU/g)									
	1		2		3		4		5	
	PCR results	Cell counts	PCR results	Cell counts	PCR results	Cell counts	PCR results	Cell counts	PCR results	Cell counts
0	0/5	2.0 ± 0.6	0/5	2.3 ± 0.4	0/5	2.9 ± 0.4	0/5	4.0 ± 0.2	2/5	5.4 ± 0.5
2	0/5	2.9 ± 0.2	0/5	3.1 ± 0.3	0/5	3.9 ± 0.9	0/5	5.1 ± 0.2	4/5	6.1 ± 0.3
3	0/5	4.0 ± 0.6	1/5	4.7 ± 0.1	2/5	5.8 ± 0.5	4/5	6.7 ± 0.2	4/5	7.6 ± 0.3
4	0/5	4.8 ± 0.4	1/5	5.3 ± 0.4	4/5	7.0 ± 0.3	4/5	7.9 ± 0.3	4/5	8.8 ± 0.1
5	1/5	5.3 ± 0.5	4/5	6.2 ± 0.7	4/5	6.6 ± 1.2	4/5	7.6 ± 0.7	-	-
6	3/5	6.1 ± 1.0	4/5	7.0 ± 0.8	4/5	7.3 ± 0.4	3/5	7.8 ± 0.8	-	-
7	4/5	7.3 ± 0.6	3/5	7.9 ± 0.5	4/5	8.5 ± 0.2	-†	-	-	-

†Not analyzed.

Table 3. *Bacillus cereus* cell counts (log CFU/g) in the fresh-cut cabbage lettuce samples and the fresh-cut baby leafy vegetable samples, and the results (the number of positive sub-samples/the number of total sub-samples) of *B. cereus* multiplex PCR kit after enrichment by tryptic soy broth (TSB) with 0.15% polymyxin at 42°C for 0 h, 2 h, 3 h, 4 h, 5 h, 6 h, and 7 h

Sample	Enrichment time (h)	The cell counts of *Bacillus cereus* (log CFU/g)						The number of positive sub-samples/ total sub-samples
		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Mean ± standard deviation	
Fresh-cut cabbage lettuce	0	3.4	3.4	3.4	3.4	3.4	3.4 ± 0.0	0/5
	2	4.1	4.2	4.2	4.3	4.3	4.6 ± 0.1	0/5
	3	6.1	6.1	6.1	6.1	6.1	6.1 ± 0.1	4/5
	4	7.3	7.0	7.0	7.0	7.0	7.1 ± 0.1	4/5
	5	7.9	8.0	8.0	7.9	7.9	7.9 ± 0.1	5/5
	6	9.2	8.9	9.0	9.0	9.1	9.1 ± 0.1	5/5
	7	8.9	8.8	8.9	8.7	8.8	8.8 ± 0.1	4/5
Fresh-cut baby leafy vegetable	0	3.3	3.5	3.4	3.4	3.3	3.4 ± 0.1	0/5
	2	4.7	4.7	4.7	4.6	4.5	4.6 ± 0.1	0/5
	3	6.0	5.9	5.9	5.9	5.7	5.9 ± 0.1	0/5
	4	7.0	7.0	7.0	7.0	6.9	7.0 ± 0.0	4/5
	5	8.1	8.0	8.2	8.1	8.0	8.0 ± 0.1	5/5
	6	8.5	8.6	8.5	8.4	8.5	8.5 ± 0.1	5/5
	7	8.5	8.2	8.9	8.2	8.7	8.5 ± 0.3	4/5

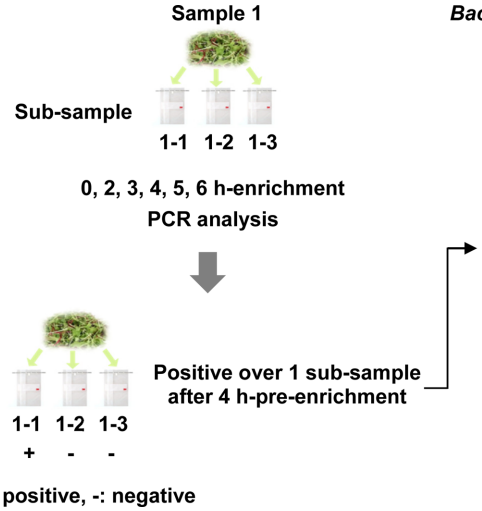
multiplex PCR kit was ~5 log CFU/g, the *B. cereus* in the enriched fresh-cut cabbage lettuce and baby leafy vegetable samples in TSB supplemented with 0.15% polymyxin B was detected at 6.7 and 6.1 log CFU/g, respectively. In addition, the number of positive samples was occasionally lower, despite longer enrichment times. These challenges arise probably due to a food matrix extracted from food components or background bacteria with longer enrichment times and uneven contamination levels in the sample; we used only 20- or 10-g portion of the sample for the enrichment. Maher et al.[12] and Hyeon et al.[13] proposed that substances may contain inhibitors that reduce the accuracy and sensitivity of the PCR analysis. Rossen et al.[14] also proposed that food components and media may also reduce the accuracy or sensitivity of the PCR reaction while Wilson[15] reported that organic or phenolic compounds are common inhibitors of PCR reactions. Kim et al.[16] concurred on the necessity of enrichment of foods to isolate bacteria



***Bacillus cereus* PCR result table of fresh-cut cabbage lettuces**

Enrichment time (h)	Initial concentration of *Bacillus cereus* (log CFU/g)				
	1	2	3	4	5
0	-	-	-	-	-
2	-	-	-	-	-
3	-	-	-	-	+
4	-	-	-	+	+
5	-	-	+	+	+
6	-	+	+	+	+
7	+	+	+		

Fig. 1. Application of rapid detection methods for semi-quantification of *Bacillus cereus* in fresh-cut cabbage lettuce through combination of enrichment and PCR detection.



***Bacillus cereus* PCR result table of fresh-cut baby leafy vegetables**

Enrichment time (h)	Initial concentration of *Bacillus cereus* (log CFU/g)				
	1	2	3	4	5
0	-	-	-	-	-
2	-	-	-	-	+
3	-	-	-	+	+
4	-	-	+	+	+
5	-	+	+	+	
6	+	+	+	+	
7	+	+	+		

About 3 log CFU/g contamination

Fig. 2. Application of rapid detection methods for semi-quantification of *Bacillus cereus* in fresh-cut baby leafy vegetables through combination of enrichment and PCR detection.

from the food matrix and background microflora for the application of PCR. Therefore, *B. cereus* can be detected in fresh-cut cabbage lettuce over the limit of detection after enrichment, and the number of positive samples was occasionally low even with longer enrichment times. Sub-samples for each sample should be analyzed to determine if the sample is *B. cereus*-positive and to address the above challenges.

To determine the minimum number of sub-samples to be analyzed in every pack of fresh-cut vegetables, 5 sub-samples were analyzed using PCR. Out of 5 sub-samples of *B. cereus* (3.4 log CFU/g) inoculated cabbage lettuce samples, 0, 0, 4, 4, 5, and 4 samples were positive after 0 h-, 2 h-, 3 h-, 4 h-, 5 h-, 6 h-, and 7 h-enrichment, respectively (Table 3). In addition, out of 5 sub-samples of baby leafy vegetables, 0, 0, 0, 4, 5, 5, and 4 samples were positive after 0 h-, 2 h-, 3 h-, 4 h-, 5 h-, 6 h-, and 7 h-enrichment, respectively (Table 3). In both kinds of samples, 4 sub-samples were the minimum numbers for a positive result in PCR analysis. Therefore, 3 sub-samples might be sufficient for a positive result if at least 1 sub-sample tests positive among 3 sub-samples. Kim et al.[17] also used 3 sub-samples for microbiological examination of the vegetable seed sprouts used, and Odumeru et al.[18] assessed the quality

of ready-to-use vegetables by analyzing 3 sub-samples. The number of sub-samples sufficient for analysis was determined to be 3 in this study as well.

Taken together, these results can lead to the development of a procedure for the semi-quantification of *B. cereus* in fresh-cut vegetables with a combination of enrichment and PCR analysis, as described in Fig 1-2. For instance, 3 sub-samples of fresh-cut cabbage lettuce samples were enriched, and 1 sub-sample was positive after 5-h enrichment. The sample then contained about 3 log CFU/g of *B. cereus* (Fig. 1). For fresh-cut baby leafy vegetables, if 1 sub-sample was positive after 4-h enrichment, the *B. cereus* cell counts were approximately 3 log CFU/g (Fig. 2).

Conclusion

Enrichment helps increase the *B. cereus* cell count, but the initial cell count after enrichment cannot be quantified. PCR analysis can identify *B. cereus* colonies on the plate, but it cannot be applied directly to the samples because its detection limit is too high. Therefore, the detection method developed in this study combines the advantages of both methods and can be used to rapidly semi-quantify *B. cereus* levels in fresh-cut vegetables.

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국문요약

신선편이채소는 주로 가열하지 않은 채로 많이 섭취되는 식품으로, 신선편이채소 섭취로 인한 식중독 사고의 위험이 지속적으로 발생하고 있다. 특히, 바실러스 세레우스는 전 세계적으로 신선편이채소에서 검출되고 있는 주요 병원성 세균이다. 본 연구에서는 신선편이채소에서 바실러스 세레우스를 신속하게 검출하기 위해 증균배양과 PCR 분석법을 조합하여 반정량 신속검출법을 개발하였다. 신선편이 양상추와 어린잎채소를 대상으로, 바실러스 세레우스 균주(KCTC1013, KCTC1014, KCTC1092, KCTC1094, KCTC3624)의 최종농도가 1, 2, 3, 4, 5 log CFU/g이 되도록 접종시킨 후 0.15% polymyxin B를 포함한 TSB 배지를 이용하여 42°C에서 0, 2, 3, 4, 5, 6, 7 시간 동안 증균배양하였다. 증균배양액 1 mL을 취하여 mannitol-egg yolk-polymyxin agar에 배양한 후 균수를 정량하였고, 1 mL의 증균배양액에서 DNA를 추출한 후 PCR 분석을 진행하였다. 또한, 신선편이채소 시료(sample)에 대한 바실러스 세레우스 검출결과를 정확하게 판단하기 위해 신선편이채소 시료(sample)의 최소분석 sub-sample수를 확인하고자, 5개의 sub-sample을 이용하여 분석하였다. 증균배양과 PCR 분석법을 이용하여 확인한 연구결과, 신선편이 양상추에 접종되어 있는 5, 4, 3, 2, 1 log CFU/g의 바실러스 세레우스는 3, 4, 5, 6, 7 시간 증균배양 후에 검출되었고, 신선편이 어린잎채소에 접종되어 있는 5, 4, 3, 2, 1 log CFU/g의 바실러스 세레우스는 2, 3, 4, 5 시간동안 증균배양한 후에 검출되었다. 또한, 신선편이채소 시료(sample)의 최소분석 sub-sample수는 3개로 확인되었다. 본 연구결과는 신선편이채소에 오염되어 있는 바실러스 세레우스의 반정량 신속검출법으로 활용될 수 있을 것이라 판단된다.

Conflict of interests

The authors declare no potential conflict of interest.

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