[S7-2]

PCR-Based Detection of Foodborne *Bacillus cereus*

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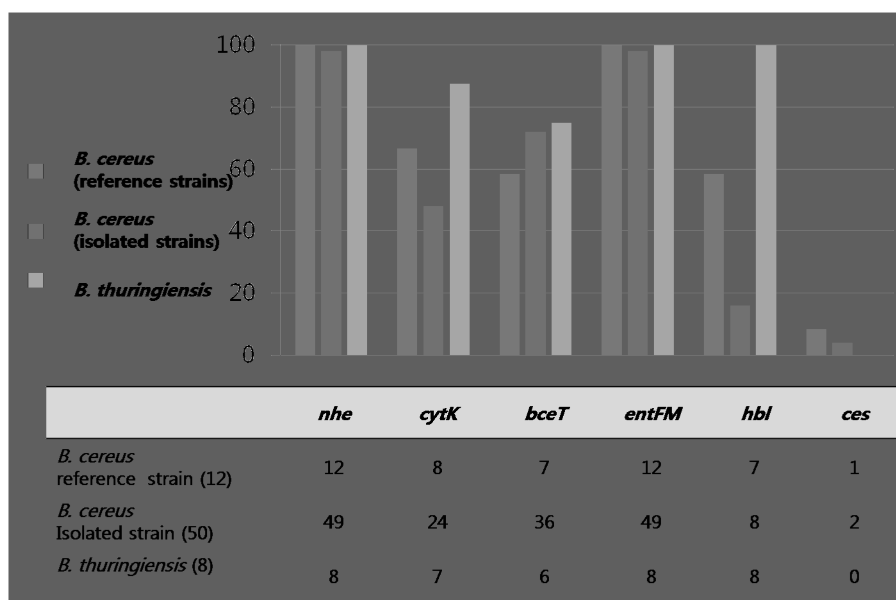
Bacillus cereus is a Gram-positive, spore-forming food pathogen commonly found in soil, dust, natural waters, and many kinds of foods. *B. cereus* causes two different types of food poisoning syndromes: diarrhea and emesis. The diarrheal type is attributed to various enterotoxins, a group of heat-labile proteins causing abdominal pain and diarrhea [1]. The emetic type is induced by the small cyclic heat-stable dodecadepsipeptide toxin cereulide, which causes vomiting [2]. Although both types of food poisoning are relatively mild, more severe cases have occasionally been reported, even involving deaths. Due to the food poisoning problem associated with *B. cereus*, there is a need to develop a reliable method for detecting and differentiating enterotoxin and emetic toxin-producing *B. cereus* strains in contaminated food. PCR has been one of the most important genetic tools for detecting pathogenic bacteria. The various toxins produced by *B. cereus* are the most popular targets for the identification of *B. cereus* strains (Fig. 1).



	nhe	*cytK*	*bceT*	*entFM*	*hbl*	*ces*
B. cereus reference strain (12)	12	8	7	12	7	1
B. cereus Isolated strain (50)	49	24	36	49	8	2
B. thuringiensis (8)	8	7	6	8	8	0

Fig. 1. Toxigenic patterns of *B. cereus* and *B. thuringiensis* strains

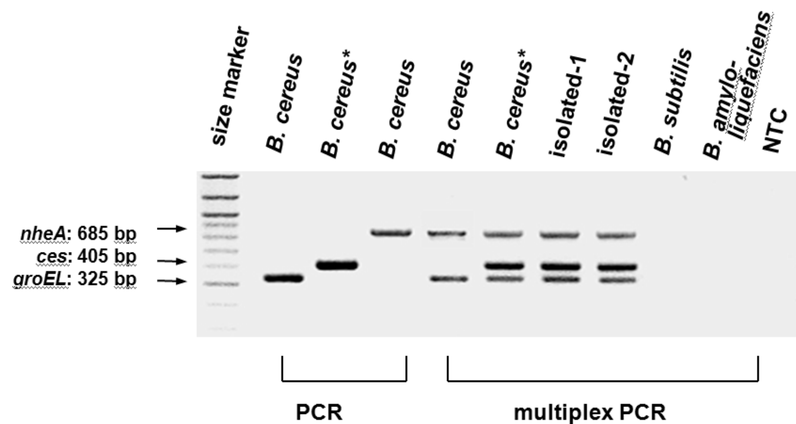


Fig. 2. *B. cereus*-specific multiplex PCR

In addition, the nucleotide sequences of nonvirulence factors such as the *groEL* gene, which encodes molecular chaperonin, are also used in the identification of *B. cereus* strains [3]. Occasionally, however, singleplex PCR may not give a reliable result due to sequence variations in target genes. In order to improve the diagnostic capacity of the PCR assay, the simultaneous amplification of two or more target sequences from templates is desirable for the detection and identification of pathogenic bacteria. Therefore, multiplex PCR assay was developed for the rapid detection and differentiation of enterotoxin-producing and emetic toxin-producing *B. cereus* strains. Three primer pairs specific to regions within genes encoding nonhemolytic enterotoxin (*nheA*), molecular chaperonin (*groEL*), and cereulide synthetase (*ces*) were used in multiplex PCR. The cereulide-producing emetic *B. cereus* showed three PCR products of 325, 405, and 685 bp for the *groEL*, *ces*, and *nheA* genes, respectively, whereas the enterotoxin-producing *B. cereus* showed two PCR products without a *ces* gene specific DNA fragment (Fig. 2). Specific amplifications and differentiations by multiplex PCR assay were obtained using 62 *B. cereus* strains and 13 strains of other bacterial species.

The detection limits of this assay for *B. cereus* from pure cultures and artificially inoculated milk without enrichment were analyzed. The detection limit of multiplex PCR assay for enterotoxin-producing strain and emetic toxin-producing strain from pure cultures were 2.4×10^1 and 6.0×10^2 CFU/tube, respectively. However, the detection limits of both strains from artificially inoculated milk were found to be 10-fold lower than those of pure cultures, respectively.

Real-time PCR offers rapid and quantitative analysis for detection of foodborne pathogens. Based on the multiplex PCR results, the real-time PCR methods with TaqMan probes targeting *groEL* and *ces* genes were developed and applied to more rapid and sensitive detection of *B. cereus* in food. The duplex real-time PCR method was shown to be able to detect as little as 3.0×10° CFU/tube of both *B. cereus* strains in pure cultures and artificially inoculated milk without enrichment, respectively (Fig. 3). Direct detection and identification of *B. cereus* in foods by duplex real-time PCR is currently progress.

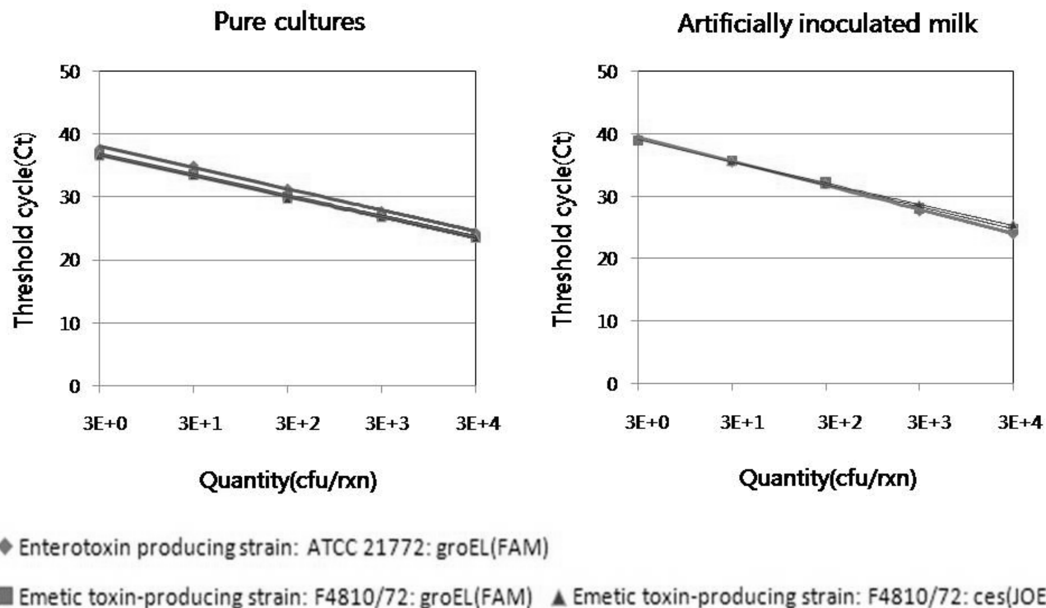


Fig. 3. Standard curves for a 10-fold serial dilution series of *B. cereus* using the duplex real-time PCR assay.

References

1. Sergeev N, Distler M, Vargas M, Chizhikov V, Herold KE, and Rasooly A. Microarray analysis of *Bacillus cereus* group virulence factors. *J Microbiol. Methods* **65**: 488-502, 2006.
2. Ehling-Schulz, M, Guinebretiere MH, Monthan A, Berge O, Fricker M, and Svensson B. Toxin gene profiling of enterotoxic and emetic *Bacillus cereus*. *FEMS Microbiol. Lett.* **260**: 234-240, 2006.
3. Chang YH, Shangkuan YH, Lin HC, and Liu HW. PCR assay of the *groEL* gene for detection and differentiation of *Bacillus cereus* group cells. *Appl. Environ. Microbiol*. **69**: 4502-4510, 2003.