Toxin-associated gene prevalence in Food-derived *Bacillus cereus* in Japan

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APCCMI2025 BANGKOK

RES-092

INTERNATIONAL UNIVERSITY OF HEALTH AND WELFARE

Introduction

Bacillus cereus is an aerobic Gram-positive spore bacillus widely distributed in natural environments such as soil, food, such as agricultural and marine products, and feed. This bacterium is known to cause food poisoning and is classified into diarrheal and emetic types. In Japan, the emetic type is considered to be the most common type. The diarrheal type is caused by the production of enterotoxin in the intestinal tract. The emetic type is caused by ingestion of the cereulide toxin. Both types of food poisoning often cause mild illness, but severe cases of liver and kidney damage and death have been reported. Foods that cause *B. cereus* food poisoning include cereal products such as rice and noodles, as well as dairy products. In this study, we investigated the contamination status of *B. cereus* and the prevalence of toxin-related genes. The sample consisted of commercially available rice balls.

Material and Method

Fifty-six samples of commercial rice balls collected from various regions of Japan were used in the study. 25 g of the samples were homogenized with 225 mL of saline solution. The sample stock solution and 10-fold dilutions were applied to standard medium and incubated at 30 ℃ for 18~24 hours. In addition, 10 mL of the sample was added to 10 mL of Heart Infusion Broth(HIB) and incubated at 30 ℃ for 18~24 hours. *Bacillus subtilis*-like colonies were isolated and biochemical properties were confirmed. Five biochemical properties were tested: lecithinase, motility, VP reaction, hemolytic activity, and glycolysis. Strains positive for lecithinase and identified as *B. cereus* group by MALDI-TOF-MS were identified as *B. cereus*. Multiplex PCR was performed on each *B. cereus* strain for a total of nine genes: hemolytic enterotoxin (*hblC, D, A*), non-hemolytic enterotoxin (*nheA, B, C*), cytotoxin (*cytK*), enterotoxin FM (*entFM*) and the emetic toxin cereulide synthesis gene (*ces*).

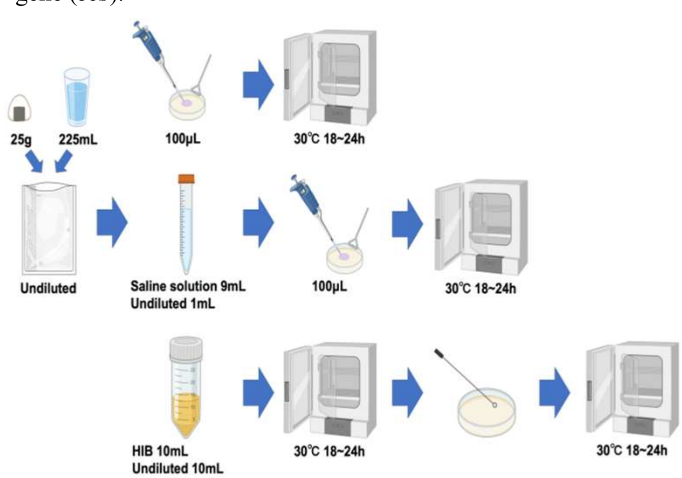

Fig.1 method

Results

B. cereus was isolated from 10 of the 56 samples, with a contamination rate of 17.8%. A total of 11 strains of *B. cereus* were isolated from 10 samples. *B. cereus* were isolated from rice balls containing a variety of ingredients, including fried rice, chicken rice, and soba rice. The toxin gene status of the 11 strains is shown in Table 1. There are four strains of *hblC*, five strains of *hblD*, four strains of *hblA*, 11 strains of *nheB*, *A*, and *C*, five strains of *cytK*, 11 strains of *entFM*, and one strain of *ces.*

Table1. Distribution of toxin genes among *B. cereus* isolates

sample	Toxin								
	Hemolysin BL complex			Non-hemolytic enterotoxin complex			Cytotoxin K	Enterotoxin FM	Cereulide
	hblD	*hblA*	*hblC*	*nheB*	*nheA*	*nheC*	*cytK*	*entFM*	*ces*
BC1	+	+	+	+	+	+	+	+	−
BC2	+	+	+	+	+	+	+	+	−
BC3	+	+	+	+	+	+	+	+	−
BC4	−	−	−	+	+	+	−	+	−
BC5	+	−	−	+	+	+	−	+	+
BC6	−	−	−	+	+	+	+	+	−
BC7	−	−	−	+	+	+	−	+	−
BC8	−	−	−	+	+	+	−	+	−
BC9	−	−	−	+	+	+	−	+	−
BC10	+	+	+	+	+	+	+	+	−
BC11	−	−	−	+	+	+	−	+	−
Total	5	4	4	11	11	11	5	11	1

+:a PCR product of the expected size was obserbed
−:no PCR product was observed

Discussion

Previous studies reported that the distribution of toxin genes in *B. cereus* isolates was approximately 40–70% for the *hbl* gene, 85–100% for the *nhe* gene, 40–70% for the *cytK* gene, and 60-100% for the *entFM* gene. In this study, the distribution was 45% for the *hbl* gene, 100% for the *nhe* gene, and 45% for the *cytK* gene, 100% for the *entFM* gene, generally consistent with previous studies. Furthermore, the *hbl* and *nhe* genes require the presence of all three genes to function as toxins. This study found that four strains possessed *hblC*, *D*, and *A*. Furthermore, all 11 strains possessed *nheB*, *A*, and *C*, and one strain possessed the *ces* gene. These strains were suggested to retain the capability to produce toxins potentially. However, it remains unclear whether possessing these genes guarantees the capability to produce toxins. Therefore, it is necessary to perform toxin quantification using LC-MS and to conduct tests using cells to confirm the production of each toxin.

Conclusion

B. cereus was isolated from commercially available rice balls, and some strains harbored toxin-producing genes. As these strains were suggested to be potential toxin-producing strains, hygiene management should be implemented to prevent *B. cereus* food poisoning.

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We have no financial relationship to disclose.