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## **ENTEROTOXIN PRODUCTION ABILITY OF *BACILLUS CEREUS* STRAINS FROM SOUTH UKRAINIAN REGION**

The objective of this study was to identify and detect emetic toxin- and enterotoxin-producing bacteria among 42 *Bacillus cereus* strains, isolated from Ukrainian food plant raw materials and products. The detection rate of *nheA*, *hblD* and *cytK* enterotoxin genes among investigated *B. cereus* strains was 100, 90,0 and 61,9%, respectively. The *ces* gene encoding emetic toxin was detected in 9,5 % of strains. Our finding revealed that *nhe* and *hbl* enterotoxins encoded by *nhe* and *hbl* genes were the major toxins among *B. cereus* tested in this study and enterotoxic type of *B. cereus* was predominant in South Ukrainian region.

Key words: emetic toxin- and enterotoxin-producing *Bacillus cereus*.

Food poisoning caused by the presence of *Bacillus cereus* in foodstuffs is recorded in almost all countries. *Bacillus cereus*, a rod shapes, gram-positive, spore-forming food pathogen, play an important role as the causative agent of diarrheal and emetic types of food poisoning [1]. The diarrheal type of food poisoning is caused by heat-labile enterotoxins such as hemolysin BL (*hbl*), nonhemolytic enterotoxin (*nhe*) and cytotoxin K (*cyt K*). The *hbl*- and *nhe*-complex both consist of three proteins (tripartite toxins). Cytotoxin K is a pore forming toxin cause necrotic enteritis.

The diarrheal syndrome, including abdominal pain and diarrheal symptoms, appears 8 to 16 h after ingestion of contaminated food. The emetic syndrome, which is characterized by nausea and vomiting within 1 to 5 h after ingestion of contaminated food, is caused by emetic toxin cereulide, a depsipeptide structurally related to potassium ionophore valinomycin, which is produced by a nonribosomal peptide synthetase (NRPS) and coded *ces* gene [2].



The objective of this study was to identify and detect enterotoxin-producing bacteria among *Bacillus cereus* strains, isolated from Ukrainian food plant raw materials and products.

### Materials and Methods

The widespread and industrially grown kinds of vegetables, fruits, berries, in particular, green peas, beetroot, tomatoes, carrots, apples, pears, plums, peaches, dill, spinach, parsley, strawberry, a number of canned and dried products, and also spices have been investigated [3]. Samples of tested materials were selected according to standardized selection rules for the average sample [4, 5].

The reference strain *B. cereus* ATCC 11778 and 42 bacilli strains isolated from food plant raw materials and products, and according to the results of previous studies, identified as *B. cereus* by studying their physiological and biochemical characteristics and fatty acid composition of cells [6].

Multiplex PCR was performed using specific primers to bacilli sequences according to Zhang et al. [7]. DNA was isolated from the samples using the SureFast® PREP Bacteria F1021 (CONGEN, Germany). The following 4 pairs of specific oligonucleotide primers for the toxicity genes were used (Table 1).

Table 1

PCR primers used in the study

Target toxin gene	Sequence (5'-3')	Amplicon size (bp)
nheA	GTTAGGATCACAATCACCGC	617
	ACGAATGTAATTTGAGTCGC	
hblD	ACCGGTAACACTATTCATGC	465
	GAGTCCATATGCTTAGATGC	
cytK	GTAACITTCATTGATGATCC	800
	GAATACTAAATAATTGGTTTCC	
cesB	ACCCATCTTGCGTCATT	154
	CAGCCAAGTGAAGAATACC	

PCR cycles are primary denaturation at 95°C for 10 min, 38 cycles of denaturation at 95°C for 1 min, annealing at 52°C for 1 min, elongation at 72°C for 1 min, final elongation at 72°C for 10 min (Thermal cycler with BioRad software, USA). Primers were chosen on the basis of literature data [7, 8] and synthesized by SPC «Simesta VAAL» (Odessa, Ukraine).



As a negative PCR control, deionized water was used to control the purity of the reagents. A visual evaluation of the size of the formed amplicons was carried out using molecular weight markers.

### Results and discussion

The detection rate of *nheA*, *hblD* and *cytK* enterotoxin genes among investigated *B. cereus* strains was 100, 83,3 and 61,9%, respectively. The *ces* gene encoding emetic toxin was detected in 9,5 % of strains (Table 2).

Table 2

**Distribution of enterotoxin genes in *Bacillus cereus* strains from different sources of south Ukrainian region**

Toxin gene	Bacillus cereus strains with enterotoxin genes (n=42)				
	Vegetables, n=14	Fruits, n=8	Canned products, n=8	Dried products, n=6	Total,%
<i>nheA</i>	14	8	8	6	100
<i>hblD</i>	12	7	8	8	83,3
<i>cytK</i>	12	4	9	1	61,9
<i>cesB</i>	3	1	-	-	9,5

The results suggest that the examined canned and dried products were free of the emetic toxin but not free of enterotoxins and the distribution of enterotoxigenic genes was significantly different among the *B. cereus* isolates from various sources.

All investigated strains of *B. cereus* were divided into 5 groups according to the presence or absence of enterotoxigenic genes (Table 3).

Table 3

**Enterotoxin genes profiles in *Bacillus cereus* strains from different sources of south Ukrainian region**

Group	<i>nheA</i>	<i>hblD</i>	<i>cytK</i>	<i>cesB</i>	No. (%) of strains (n=42)
I	+	+	+	+	2 (4,7%)
II	+	+	+	-	7 (16,6%)
III	+	+	-	-	9 (21,4%)
IV	+	-	+	-	8 (19,0%)
V	+	-	-	-	16 (38,1%)

Only 2 strains from group I (4,7%) have the ability to cause both diarrheal and emetic type of food poisoning. Group II (7 strains, 16,6%) contained the *nheA*, *hblD* and *cytK* enterotoxin genes, but no *cesB* encoded emetic toxin.



Group V was the major patterns and represented 38,1% strains. The reference strain *B. cereus* ATCC 11778 has all the tested genes of toxicity.

These finding revealed that nhe and hbl enterotoxins encoded by nheA and hblD genes were the major toxins among *B. cereus* investigated in this study and enterotoxic type of *B. cereus* was predominant in South Ukrainian region.

Our research of contamination of enterotoxin-producing strains *Bacillus cereus* raw materials from Ukrainian region are original, although these results are good agreement with food products investigation from Mexican, Dutch and Korean regions [1, 2, 8].

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