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The materials reflect the content of the conference reports, which outline the results and prospects of research in various fields of microbiology and biotechnology, in which young scientists, PhD students, and students.

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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-producting 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-producting *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 entrotoxin (nhe) and cytotoxin K (cyt K). The hbl- and nhe-complex both consist of three proteins (tripatite toxins). Cytotoxin K is a pore forming toxin cause necrotic enterotitis.

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 causes 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-producting 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	GTAACTTTCATTGATGATCC	800
	GAATACTAAATAATTGGTTTCC	
cesB	ACCCATCTTGCGTCATT	154
	CAGCCAAGTGAAGAATACC	

PCR cycles are are primary denaturation at 95° C for 10 min, 38 cycles of denaturation at 95 ° C for 1 min, annealing at 5,2° 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,%
nheA	14	8	8	6	100
hblD	12	7	8	8	83,3
cytK	12	4	9	1	61,9
cesB	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 enterotoxic genes (Table 3).

Table 3

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

Group	nheA	hblD	cytK	cesB	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 to 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-producting 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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