Identification of bacillary microbial contaminants and food poisoning agents from ukrainian plant raw materials and products

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Abstract

Introduction. The characteristics of biological contaminants occurring in plant food products, such as foodborne infections and poisonings, causative agents of spoilage, accelerated indication of potential danger to the consumer are of scientific and practical importance.

Materials and methods. A row of widespread and industrially grown kinds of vegetables, fruits, berries and a number of canned and dried products and spices were investigated. Morphological, cultural and biochemical properties of the isolated cultures were studied by conventional methods. Polymerase chain reaction (PCR) was performed using group-specific and species-specific primers to bacillary sequences with electrophoresis of PCR products in 1.5% agarose gel.

Results and discussion. Bacillary microbial contaminants and potential causative agents of food poisoning and food spoilage, which are common in industrially processed types of vegetable raw materials (vegetables, fruits, berries and products of their processing) in Ukraine, have been investigated. The dominance of the *subtilis-licheniformis* morphotypes of the order *Bacillales* among the detected rod-shaped spore-forming microorganisms is a feature of the Ukrainian vegetative raw materials. The composition of microbiota of various types of vegetable raw materials and products of their processing were studied by the complex of their phenotypic and molecular-genetic properties. The long duration and potential inaccuracy of identification of aerobic and facultative-anaerobic spore-forming bacteria by the complex of their phenotypic properties has been showed. The method of preparation of food samples and PCR with group-specific and species-specific primers for speeding-up diagnostics of *B. cereus, Paenibacillus polymyxa, P. macerans* strains in samples have been tested. Contamination of samples of plant raw materials and products of their processing by epidemiologically significant microorganism *B. cereus* were examined, and showed levels from 16.7% in fresh fruits to 72.7% in spices from the total number of samples.

Conclusions. The bacillary microbial contaminants were identified and a speeded up method of food samples preparation for PCR to detect regulated bacillary microorganisms that affect product safety was tested.

Introduction

The assessment of food safety in modern conditions is relevant all over the world [1,2], as an important characteristic of the quality of nutrition is becoming increasingly important due to increasing pollution [3], and microbiological hazards as a priority in assessing the degree of risk are due to the presence of regulated microorganisms in food. Being criterial in the system of indicators of safety and quality of food, microbiological contaminants also characterize the suitability of products for use [4, 5]. Besides, the qualitative and quantitative composition of the microorganisms of raw materials, along with its biochemical properties, determines the types, methods and regimes of technological processing [6,7].

The deterioration of the man-made environment associated with urbanization, climatic and geographic and environmental conditions of man, reducing its immunoreactivity and affecting the individual microecosystems, actualizes the need for strict control of food safety and the development of modern accelerated methods for detecting microorganisms. When processing vegetable raw materials, particularly canned food, the quality and safety of the finished product depends on the quality of the processed raw materials and is determined by the absence of microorganisms and their toxins that are dangerous to human health or changing their nutritional value [1,8,9]. In the Codex Alimentarius CAC / GL 21 document, a number of EU policy documents - the report of the EU Commission, EU guidance document 2073 - and the documents of the Federal Food and Drug Administration provide general information on the principles for the development and application of microbiological criteria for different types of food products [10, 11, 12].

Among the aerobic and facultative anaerobic spore-forming bacteria of the *Bacillales* order [13], the genus *Bacillus* is one of the largest and most common, currently includes 268 species and 7 subspecies [14], among which are the causative agents of human foodborne illness and food spoilage [15-18]. The study of the quantitative and qualitative composition of the microbial population of fruits, vegetables, berries, and especially their thermally stable species, underlies the development of technological solutions for preserving the native properties of plant raw materials before processing and guaranteeing product safety for the consumer.

The abundance of microorganisms that make up the microbiota of plant raw materials, the duration and inaccuracy of the identification of individual species of bacilli by traditional methods of research, actualize the development of speeded up methods of detecting pathogenic species that affect product safety. Therefore, the characteristics of biological contaminants occurring in plant food products – agents of foodborne infections and poisonings, accelerated indication of potential danger to the consumer, development of speeded up and reliable methods for controlling the safety of products are of scientific and practical interest.

Thus, the *goal of the research* is the identification of aerobic and facultative anaerobic spore-forming bacteria of raw materials - fruits, vegetables, berries and products of their processing, and an accelerated assessment of the safety of plant products in relation to bacillary microbial agents of food poisoning of humans and spoilage of products.

Materials and methods

Microorganisms

Researches of 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. Samples of raw materials were selected according to standardized selection rules for the average sample [9, 20] immediately after the raw material was delivered for processing, the processed products in packed, dried or canned form - after inspecting batches, paying special attention to possible defective samples. Each sample was tested in triplicate.

Bacterial strains used in this study were obtained from the National Collection of Type Cultures from Institute of Microbiology and Virology D.K. Zabolotny of NAS of Ukraine, State institution "Ukrainian Centre for Disease Control and monitoring of the Ministry of Health of Ukraine", Scientific Research Institute of preventive toxicology and disinfection of the Ministry of Health of Ukraine, Collections of Type Cultures of Odesa National Academy of Food Technologies and Odesa I. I. Mechnikov National University and were used as a control strains. They are *Bacillus cereus* ATCC 11778, *B. cereus* ATCC 10702, *B. licheniformis C*, *Paenibacillus macerans* B 5803^T, *P. polymyxa* B 5760^T, *B. coagulans* B 5850^T, *Geobacillus stearothermophilus* UCM B 718, and 117 strains isolated from food samples of edible raw material and products.

Determination of phenotypic properties

For the analysis, flushing or shredded samples of an average raw material sample were used which were heated for 20 minutes at a temperature of (80 ± 1) °C and after cooling to room temperature were plated onto meat-peptone agar (MPA) and incubated at a temperature of 30 ± 1°C for 24-48 hours [9, 21]. Samples of dried or canned products were examined without additional heating. To account for the total number of bacteria in the raw material, the samples were also inoculated without heating. Mesophilic aerobic and facultative anaerobic bacteria (MAFAnM) were taken into consideration for inoculation for MPA.

Morphological, cultural and biochemical properties of the isolated cultures were studied by conventional methods on the basis of: the growth pattern on solid and liquid nutrient media (MPB, MPA, MPA enriched with starch, nitrates, etc.), saccharolytic properties – by inoculation of semi-liquid Giss' media, proteolytic properties by inoculation of milk, and meat-peptone gelatin (MPG), determination of indole – by paper indicator impregnated with oxalic acid solution, catalase – by reaction with hydrogen peroxide, production of acetoin – by reaction with egg yolk, hemolytic activity – by the ability of microorganisms to break hemoglobin by direct inoculation of culture on blood agar [7, 9, 20 – 22]. A quantitative characteristic was established as the proportion (%) of bacillary species of microorganisms from the total number of detected contaminants.

DNA extraction and PCR

PCR was performed using group-specific and species-specific primers to bacilli sequences according to Park et al. [23]. DNA was isolated from the samples using the SureFast® PREP Bacteria F1021 (CONGEN, Germany). The composition of the mixture for PCR: 10x PCR buffer (reaction buffer for amplification, optimized for highly specific PCR, designation 10x implies dilution factor by other additive components of the reaction mixture) - 2 μl, $MgCl_2$ solution with a molar concentration of 0.05 mol/l - 0, 8 μl, a solution

of dNTPs with a molar concentration of 0.0025 mol/l of 1.6 µl, a Taq polymerase solution with an enzymatic activity of 5 U/µl is 0.4 µl. Reagents from Fermentas (Latvia) were used. The supernatant containing DNA was introduced into the reaction mixture in a volume of 5 µl.

The following pair of group-specific oligonucleotide primers for the *groEL* gene was used, which is characteristic for all representatives of the *Bacillus cereus* group and 3 pairs of species-specific primers for individual microorganisms, namely:

to *B. cereus* group BCGSH - 1F GTGCGAACCCAATGGGTCTTC *groEL*
BCGSH - 1R CCTTGTTGTACCACTTGCTC;
to *B. thuringiensis* type BTJH - 1F GCTTACCAGGGAAATTGGCAG *gyrB*
BTJH - 1R ATCAACGTCGGCGTCGG;
to *B. cereus* type nhe A F AAGGCGAATGTACGAGAGTGG *nhe A*
nhe A R CTTCTCTCGTTTGACTATCTGCAG;
to *Paenibacillus polymyxa* type 29Pp F GAGCGGGGTTGATTAGAAGC
179Pp R CTTTCCTCCTTCTCCCATGC ;
to Paenibacillus macerans type MAC 1 ATCAAGTCTTCCGCATGGGA
MAC 2 ACTCTAGAGTGCCCAMCWTT.

PCR cycles are primary denaturation at 94 °C for 5 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 63 °C for 30 s, elongation at 72 ° C for 30 s, final elongation at 72 °C for 5 min (Thermal cycler with BioRad software, USA). Primers were chosen on the basis of literature data [23–25] and synthesized by SPC "Simesta VAAL" (Odesa, 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.

Electrophoresis of PCR products was carried out in a 1.5% agarose gel. Trisacetate buffer was used (Equipment for electrophoresis of PCR products from BioRad, USA). DNA was stained with ethidium bromide (0.5 µg/ml) and photographed with a video system (BioRad, USA) under UV light (wavelength 312 nm).

Results and discussion

Phenotypic identification of bacillary contaminants

The composition of microorganisms of food raw materials and products characterizes both the possibility of epidemiological risk and high-quality products. Table 1 presents a description of the morphophysiological and biochemical properties of 69 isolated strains of bacilli, the presence of spores in which does not modify the vegetative cell, and utilizing arabinose, mannitol and xylose with acid formation without gas (6 morphotypes). These cultures had the following general properties: medium-sized sticks (0.6-0.8) x (1.5-3.0) - (1.0-1.2) x (3.5-5.0) µm with elliptical spores located centrally and not exceeding the size of the cells. Also of them are gram positive; 25 isolates had pronounced mobility in diurnal culture.

Table 1

Description of the acid-forming bacilli of vegetable raw material and products

Characteristics	Properties of bacilli by morphotypes					
	I	II	III	IV	V	VI
Number of isolates taken for identification	20	16	6	12	10	5
Cell sizes, microns	(0,7–,8)× (2,0–3,0)	(0,6–0,8)× (1,5–2,0)	(0,6–0,7)× 2,0–2,5)	(1,0–1,2)× (3,0–4,0)	(1,0–1,2)× (3,0–4,0)	(1,2–1,5)× (2,5–3,0)
Growth on MPA in anaerobic conditions	–	+	–	+	+	–
Hydrolysis of starch	+	+	–	+	+9 cultures -1 culture	+
Reduction of nitrates	+	+	–	+	+	+
Decarboxylation of tyrosine	–	–	–	+	+8 cultures ±2 cultures	+
Hemolytic activity	–	–	–	+	+	–
Lecithinase activity	–	–	–	+	+	–
The reaction of Voges-Proskauer (production of acetoin)	+	+	+	+	+	–
Production of acid from arabinose, xylose, mannitol	+	A+ G±	+	–	–	+3 cultures –2 cultures
Intended view	*Bacillus subtilis*	*Bacillus licheniformis*	*Bacillus pumilis*	*Bacillus cereus*	*Bacillus thuringiensis*	*Bacillus megaterium*

All cultures have an aerobic type of respiration (catalase-positive), but 38 of them also showed the ability to grow on MPA under anaerobic conditions. Of the other common properties, all isolated cultures with varying degrees of intensity exhibited the ability to liquefy gelatin, hydrolyse casein, assimilate glucose, lactose, sucrose to produce acid without gas production, but only 45 of them developed on media with mannitol, xylose or arabinose. To the utilization of tyrosine, the ability was detected in 27 isolated cultures, for two it was not established reliably. With the exception of six isolates, all reduced nitrates to nitrites. Twenty-two cultures showed lecithinase activity.

Determination of the proportion of isolated acid-forming bacilli from the identified (%) allows them to be arranged in descending order in the following order: *Bacillus subtilis-Bacillus licheniformis* > *Bacillus cereus* > *Bacillus megaterium* > *Bacillus pumilis* ≥ *Bacillus thuringiensis*.

According to the totality of the morphological and cultural features of the isolated cultures of the first and second morphotypes studied by us, it can be concluded that, with a common coincidence of most of the individual indices, they differ among themselves by few (the relief of the colonies - representatives of the I morphotype on the MPA formed small grayish shiny colonies and II morphotypes - blurred; representatives of the first

morphotype, when sown with a prick, gave crater-like liquefaction of gelatin, representatives of the second morphotype were saccular, and representatives of the I and II morphotypes opacity and thin film were formed on MPB, but in the second case, the broth was cleared, the representatives of the first morphotype alkalized the milk during peptonization) and can be combined into the group *subtilis-licheniformis*. These cultures accounted for the largest proportion of bacilli found on raw materials. It is these microorganisms that most often constitute the permissible aerobic microbiota of heat-treated benign products intended for long-term storage [20].

From Table 1 description it becomes created that bacillus of the *subtilis-licheniformis* group, six cultures from the third morphological group were distinguished by smoother, whitish shiny colonies on MPA growing into a substrate, formation of a thin film on the MPB, and opacity, peptonization of milk without clotting; crater-like liquefaction of gelatin during inoculation in a stalk, lack of amylase and tyrosinase activity and the ability to reduce nitrates. Presumably they were attributed to the species *Bacillus pumilis*, whose number was insignificant and amounted to no more than 10.8% of the total number of bacilli that contaminated the investigated raw materials.

In the fourth group of cultures, smooth grayish-white colonies formed on the MPA, caused an opacity of the MPB and the formation of a sediment, did not change the kind of milk, did not liquefy gelatin when planted with a prick, forming a brilliant coating on the surface. They split maltose, did not split mannitol. In the early stages of growth on glucose agar, the cells contained fat globules. Disputes formed quickly. All cultures exhibited lecithinase activity on the yolk agar, formed acetoin and characteristic ruby colonies on salt agar with 2,3,5-triphenyltetrazolium chloride, as well as indole, which confirmed their difference from *B. pumilis* and microorganisms of the *subtilis-licheniformis* group. This allowed us to define them as *B. cereus*. On the types of plant raw materials studied, *B. cereus* varieties comprised between 8.5 and 29% of the total number of bacilli.

Colonies of group V bacilli are roundish, greyish-white, with a pasty consistency, a matte surface, like *B.cereus*, with a slightly-wavy margin. Presumably, this group can be formed by strains of *B. thuringiensis*. On the investigated vegetables, *B. thuringiensis* species were small, but prevailed on parsley and spices.

An important reference point in the identification of group VI bacilli was the size, cell structure, folded macrorelief of the colony, which differentiate them from the species described above. Colonies on MPA are round, thick, convex, whole, shiny, slimy. With the age of the culture, the substrate is colored brown. On the MPB, the growth is meager in the form of haze, forming a greyish surface coating on the gelatinous media, when planting with a stab in the column - liquefaction in the form of a crater. Milk does not roll, peptonizes. In old cultures, the growth in MPA revealed fat. Representatives of the sixth group were identified as *B. megaterium*. Reactions of tyrosine cleavage and reduction of nitrates varied depending on the age of the culture. The heat-resistant strains of these bacilli in the samples were also small: 4–14%.

The bacilli described in Table 2, are gram-positive mobile rods whose spores are larger in diameter than the thickness of the cells and are subterminal or terminal. They form catalase, but are able to grow on MPA under anaerobic conditions, and also hydrolyze starch, casein, reduce nitrates to nitrites, do not form indole, lecithinase and tyrosinase. In contrast to the bacilli described in Table. 1, when cultivated on media with arabinose, xylose and mannitol form a gas along with the acid.

The group of bacilli VII is made up of microorganisms that grow poorly on MPA with the formation of thin round beige widespread colonies. They cause turbidity of the MPB and form a mucous precipitate. The Gram-staining of cells during cultivation on different

media showed variability. These bacilli do not decarboxylate tyrosine and do not form acetylmethylcarbinol, liquefy gelatin. On a gelatinous medium, a weak surface coating is formed, they do not cause liquefaction during seeding. Starch hydrolyzed completely - to mono- and disaccharides. Milk coagulate with the formation of gas, utilize glucose, lactose, maltose to form acid. The complex of the revealed properties of this group basically coincides with the description of bacilli of the species *B. macerans* (formerly called *B. aerosporus*), which are currently reported to be of the genus *Paenibacillus* [26].

Table 2

Description of the acid- and gas-forming bacilli of vegetable raw material and products

Characteristics	Properties of bacilli by morphotypes		
	VII	**VIII**	**IX**
Number of isolates selected for identification	15	19	14
Cell sizes, microns	(0,5–0,6)× (3,0–4,0)	(0,6–0,7)× (2,0–3,5)	(0,7–1,0)× (2,0–3,0)
Hydrolysis of casein	–	+	+
Gelatin liquefying	+	± weak reaction	±
Production of acetoin	–	+	–
Decarboxylation of tyrosine	–	–	–
Intended view	*Paenibacillus macerans*	*Paenibacillus polymyxa*	*Bacillus circulans*

A distinctive feature of the *Bacillus* group VIII is the formation of mucus on dense and liquid substrates and slow liquefaction of gelatin. On MPA form grayish shiny large colonies, on the MPB – turbidity, sediment, surface film of grayish color. Milk does not coagulate, does not form an indole. The starch is hydrolyzed, gelatin liquefies slightly (bag-like liquefaction). These bacilli can presumably be attributed to varieties of *P. polymyxa*.

Group IX was made up of bacilli, which form thin spreading colonies on the surface of the MPA. Causes mild turbidity of the MPB and mild acid formation in milk (slow coagulation). On gelatinous media grow in the form of a slight surface coating, when inoculation with a prick growth was absent. Glucose, lactose, sucrose is digested with the production of acid. Three isolates in this group after growing on different substrates stained Gram variably, the rest - positively. By the type of respiration they are classified as facultative anaerobic microorganisms. They do not form acetoin, slowly dilute gelatin, hydrolyze casein. According to most of the characteristics, the description corresponds to *B.circulans*, a number of strains which belong to the genus *Paenibacillus* [27]. This species is considered mesophilic, but the literature notes the presence of thermophilic variants [1].

Detection of bacilli VII-IX morphotypes in raw materials draws attention to the need for their control in packaged products and after heat treatment (in particular, preserves, canned food) as potential causative agents of bomb damage.

Acid-forming and gas-forming bacilli on the studied raw materials are represented by a relatively small amount – from 2–4% on strawberries to 15% on green peas from the total number of allocated bacilli.

It should be noted that the morphophysiological, cultural and biochemical properties of the studied cultures did not always show convincingly. On different media, some R-form colonies transformed into S-form colonies, which made it difficult to identify themselves by

culture and tinctorial characters. Identification difficulties prevented the introduction of the characteristics of some crops into tables and clearly determined the proportion of isolates studied in the total number of bacilli found on the raw materials examined. As studies have shown, the precise identification of bacillary species of microorganisms by classical methods is not only time-consuming, laborious, but often difficult to accurately identify.

PCR detection of B. cereus in food samples

Since the microorganisms of the *B. cereus* group cause foodborne illnesses and are potentially enterotoxigenic for humans, the ability to rapidly detect *B. cereus* in food is critical [1, 5–7, 23, 28].

To choose more efficient pretreatment method of raw samples and products for detection of enterotoxin-producing *B. cereus*, samples were inoculated with the culture of each test strain of bacilli and further experiments with DNA release from strains *B. cereus* ATCC 11778, *B. cereus* ATCC 10702, *B. cereus* UCM B 5650 and *B. cereus* UCM B 5671 were carried out in three variants: inoculated bacteria samples without pretreatment (wells 7, 9, 10), and the samples previously centrifuged to remove organic residues and filtered through «Millipore» nitrocellulose membrane filters (wells 1, 4), and the samples centrifugated twice in developed modes (wells 3, 11, 12, Figure 1).

It was revealed that pre-treatment of samples is desirable for the detection of toxin-producing *B. cereus* in plant raw materials and products using PCR: on the electrophoregram the amplicons that were formed in the case of filtering were more clear, however, preliminary preparation with double centrifugation was most effective (Figure 1).

Thus, the method of preliminary treatment of samples of plant raw materials and products with double centrifugation: the first - to remove the residues of organic substances of the product and the second for the concentration of microorganisms. Sampling regimes have been submitted for priority and they have been used for further research.

To confirm the specificity of PCR, the *B. cereus* strain UCM B 5671 was tested with the nhe primer to the enterotoxigenic gene *nhe A* (well 17). The size of the amplicon formed was 553 bp. Thus, by the PCR method, the ability to form an amplicon at 400 bp to the gene *groEL*, which is characteristic for all representatives of *B. cereus* group.

B. cereus causes diarrhea and emetic syndromes, producing various extracellular toxins, including the three main types of enterotoxins, namely hemolysin BL (*hbl*), nonhemolytic enterotoxin (*nhe*) and cytotoxin K (*cyt K*) [13].

Among the strains of *B. cereus*, enterotoxigenic genes *hbl A, nhe A, cyt K* and *Fm* (enterotoxin FM) were widely spread. However, we selected only the *nhe A* gene for PCR, given its greatest prevalence and detectable toxicity, which is associated with a major role in food poisoning. The polymerase chain reaction with specific primers nhe A F and nhe A R, matched to the site of the *nhe A* gene, confirmed the belonging of all tested collection strains of *B. cereus* to the enterotoxigenic species of *B. cereus*, whereas in PCR analysis of the DNA of the collection species *G. stearothermophilus* and *P. polymyxa* and in negative control (PCR mixture without DNA), no amplification products were detected. The size of the amplicons was 553 bp, which indicated the proper specificity of the PCR.

PCR results with product samples containing different combinations of bacterial strains using specific species-specific primers are shown in Figure 2.

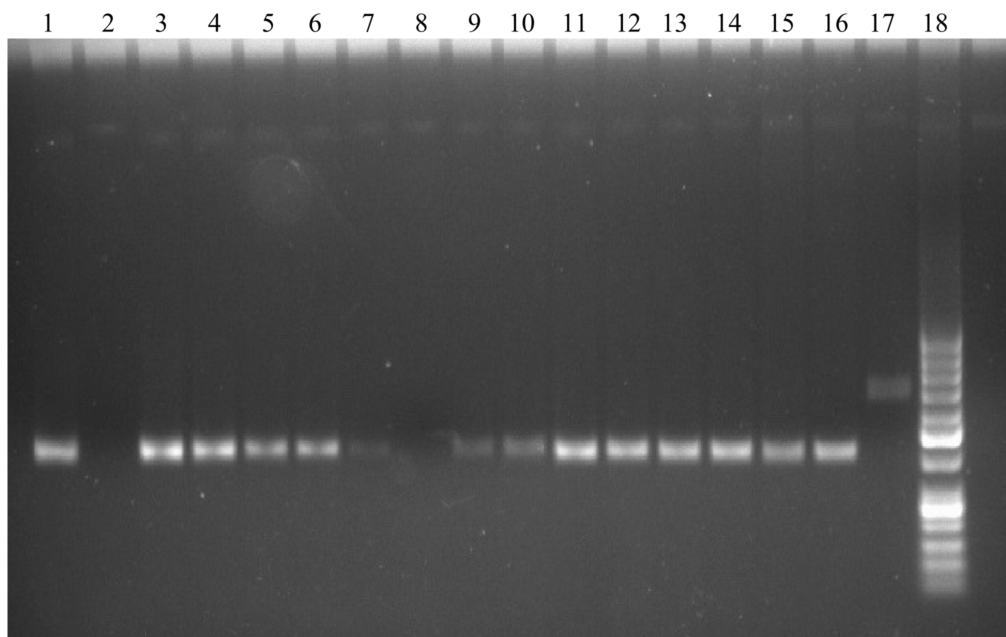

Figure 1. Electrophoregram of PCR products with DNA of strains of *Bacillus* spp. with a pair of specific oligonucleotide primers to the gene *groEL*:

1. *B. cereus* UCM B 5671 (using a membrane filter)
2. Negative PCR control;
3. *B. cereus* ATCC 11778 (using centrifugation)
4. *B. cereus* ATCC 10702 (using centrifugation)
5. *B. cereus* UCM B 5650 (using a membrane filter)
6. *B. cereus* UCM B 5671 (using a membrane filter)
7. *B. cereus* UCM B 5671 (without filtration)
8. Do not inoculate bacteria from cans;
9. *B. cereus* ATCC 11778 (without filtration)
10. *B. cereus* ATCC 10702 (without filtration)
11. *B. cereus* P90-1 (using centrifugation)
12. *B. cereus* P90-4 (using centrifugation)
13. *B. cereus* P90-9 (using centrifugation)
14. *B. cereus* L-3 (using centrifugation)
15. *B. cereus* L-6 (using a membrane filter)
16. *B. cereus* L-7 (using a membrane filter)
17. *B. cereus* UCM B 5671 with the primer nhe A;
18. Molecular weight markers (pBR322 / BsuRI, Fermentas).

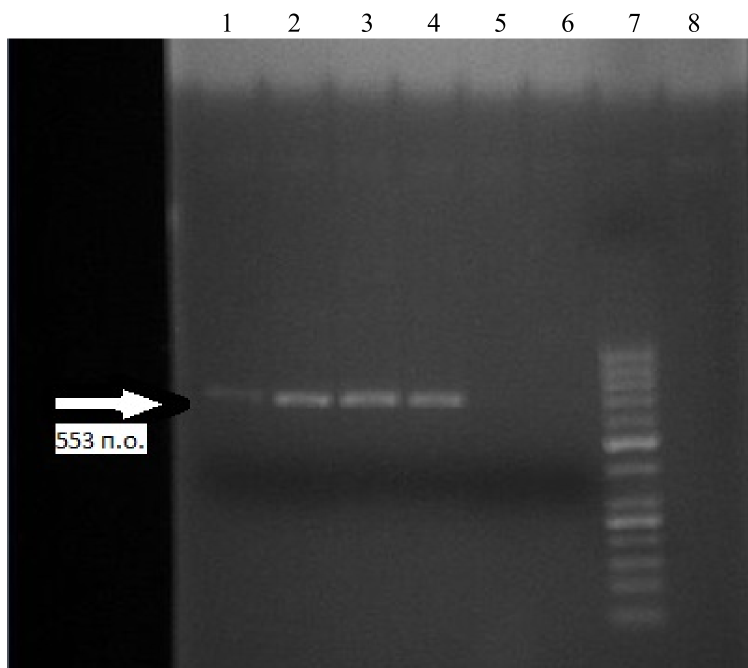

Figure 2. Electrophoregram of PCR products with DNA strains of *B. cereus, P. polymyxa, P. macerans, E. coli, S. aureus, C. perfringens, G. stearothermophilus* species with a pair of specific oligonucleotide primers to the *nhe A* gene:

1. A mixture of *B. cereus* strains (*B. cereus* ATCC 11778, *B. cereus* ATCC 10702, *B. cereus* UCM B 5650, *B. cereus* UCM B 5671);
2. A mixture of strains of *B. cereus*, *P. polymyxa* and *P. macerans* (*P. macerans* B 5803^T, *P. polymyxa* B 5760^T, *B. cereus* ATCC 11778, *B. cereus* ATCC 10702, *B. cereus* UCM B 5650, *B. cereus* UCM B 5671);
3. A mixture of *B. cereus* and *P. polymyxa* strains (*P. polymyxa* B 5760^T, *B. cereus* ATCC 11778, *B. cereus* ATCC 10702, *B. cereus* UCM B 5650, *B. cereus* UCM B 5671);
4. A mixture of *B. cereus* and *P. macerans* strains (*P. macerans* B 5803^T, *B. cereus* ATCC 11778, *B. cereus* ATCC 10702, *B. cereus* UCM B 5650, *B. cereus* UCM B 5671);
5. *C. perfringens* and *G. stearothermophilus* UCM B 718;
6. *Staphylococcus aureus* ONU 223;
7. Molecular weight markers (pBR322 / BsuRI, Fermentas);
8. *Escherichia coli* UCM B 906.

Primers nhe A F and nhe A R formed an amplicon sized 553 bp in PCR with *B. cereus* strains, primers 29Pp F, 179Pp R formed an amplicon with a size of 150 bp with strains of the species *P. polymyxa*, primers MAC 1, MAC 2 formed an amplicon with a size of 100 bp with strains of the species *P. macerans*. In PCR using the DNA of gram-negative bacteria *Escherichia coli* UCM B 906 and gram-positive bacteria *Staphylococcus aureus* ONU 223, *C. perfringens* and *G. stearothermophilus* UCM B 718, which were conducted to verify the authenticity of the results of detection of natural contamination of specimens by food poisoning and product damage agents, no amplification product was obtained. The results of detection of strains of the *B. cereus* species by the presence of the *nhe A* gene in the studied plant products are shown in Table 3.

Table 3

***B. cereus* contamination of plant raw materials and products of their processing**

Product type	Number of samples, n	Number of samples that contain *B. cereus*	Proportion of contaminated samples, %
Fresh fruit	12	2	16,7
Fresh berries	9	3	33,3
Fresh vegetables	34	21	61,8
Canned food with signs of spoilage	9	4	44,4
Dried vegetable mixes	16	7	43,7
Spices	11	8	72,7
Dried herbs	14	10	71,4
Vegetables boiled in vacuum polymer bags	12	2	16,7

Comparing the results with those given for vegetables from the city of Mexico, it is possible to note practically the same trends of detection of *B. cereus* for most types of fresh vegetable raw materials – 61.8% and 57% for the Ukrainian and Mexican regions, respectively [29]. And as noted by Valero et al. [30] in Spain, all samples of fresh raw materials - peppers, cucumbers, tomatoes, carrots, zucchini, onions - were contaminated with *B. cereus*. This, according to INFOSAN [31], leads to an increase in the incidence of foodborne illness.

Conclusions

Bacillary microbial contaminants and agents of food poisoning of plant raw materials and products of the Ukrainian region were identified. The composition of microbiota of various types of vegetable raw materials – vegetables, fruits, berries – and products of their processing were studied. A feature of plant raw materials of Ukraine is the dominance of rod-shaped spore-forming microorganisms of the *Bacillales* order of the group *subtilis-licheniformis*. To accelerate the indication of the potential hazard of products for consumers, a method for preparing food samples was tested and PCR with group-specific and species-specific primers was performed in order to diagnose strains of *B. cereus, P. polymyxa* and *P. macerans* microorganisms in samples. The contamination of samples of plant raw materials and products of their processing with epidemiologically significant microorganism *B. cereus* were examined, and showed levels from 16.7% in fresh fruits to 72.7% in spices from the total number of samples.

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Анотації

Харчові технології

Ідентифікація бацилярних мікробних контамінантів і збудників харчових отруєнь в українській рослинній сировині і продуктах

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Вступ. Характеристика біологічних забруднень рослинних харчових продуктів – збудників харчових інфекцій і отруєнь, збудників псування та прискорене визначення потенційної їх небезпеки для споживача мають наукове і практичне значення.

Матеріали і методи. Досліджували поширені і промислово вирощувані види овочів, фруктів, ягід, ряд консервованих і сушених продуктів, а також спецій. Морфологічні, культуральні та біохімічні властивості виділених культур вивчали загальноприйнятими методами. Полімеразну ланцюгову реакцію (ПЛР) проводили з використанням групо- і видоспецифічних праймерів до послідовностей бацил та електрофорезу продуктів ПЛР в 1,5% агарозному гелі.

Результати і обговорення. Досліджені бацилярні мікробні контамінанти, потенційні збудники харчових отруєнь і псування продуктів для промислово поширених в Україні видів рослинної сировини – овочів, фруктів, ягід і продуктів їх переробки. Особливістю рослинної сировини України є домінування морфотипів *subtilis-licheniformis* серед виявлених паличковидних спороутворюючих мікроорганізмів порядку *Bacillales*. Склад мікробіоти різних видів рослинної сировини і продуктів її переробки було досліджено за комплексом їх фенотипових і молекулярно-генетичних властивостей. Встановлено, що ідентифікація аеробних і факультативно-анаеробних спороутворюючих бактерій за комплексом їх фенотипових властивостей тривала і не завжди дозволяє точно визначити вид мікроорганізмів. Апробовано методику підготовки зразків харчових продуктів і проведено ПЛР з групо- і видоспецифічними праймерами з метою прискореної діагностики в зразках штамів *B. cereus*, *Paenibacillus polytyxa*, *P. macerans*. Визначено контамінованість зразків рослинної сировини і продуктів її переробки епідеміологічно значимим мікроорганізмом *B. cereus*, яка становить від 16,7% для свіжих фруктів до 72,7% для спецій і прянощів від загальної кількості досліджених зразків.

Висновки. Ідентифіковано бацилярні мікробні контамінанти та апробовано прискорену методику підготовки зразків харчових продуктів з ПЛР для індикації регламентованих бацилярних мікроорганізмів, які впливають на безпеку продукції.

Ключові слова: *бацила, харчовий продукт, безпека, B. cereus, ПЛР, фенотип.*