

PRETREATMENT OF SAMPLES FOR DETECTION OF ENTEROTOXIN-PRODUCING *BACILLUS CEREUS*

Sergieieva Zh. ¹, Pylypenko I. ², Pylypenko L. ², Yamborko G. ¹

¹Department of Microbiology, Virology and Biotechnology, Odesa I. I. Mechnikov National University, Dvoryanska str., 2, 65082 Odesa, Ukraine

²Department of Biochemistry, Microbiology and Physiology of Nutrition, Odesa National Academy of Food Technologies, Kanatna str., 112, 65039, Odesa, Ukraine

E-mail: sergeevazh@gmail.com

The *Bacillus cereus* group includes important spore-forming bacteria that present spoilage capability and may cause foodborne diseases. *Bacillus cereus* is responsible for several outbreaks of foodborne diseases due to its emetic toxin and enterotoxin. Enterotoxins, cytotoxin K (CytK), nonhemolytic enterotoxin (Nhe), and hemolysin BL (Hbl), have been recorded in several diarrheal cases due to food poisoning from *B. cereus*. These microorganisms are traditionally evaluated in food using culturing methods, which can be laborious and time-consuming, and may also fail to detect bacteria in a viable but nonculturable state.

The objective of this study was to develop a method for isolating microorganisms for rapid and accurate method that uses a multiplex PCR for the detection of cells of enterotoxin-producing *B. cereus*. The inclusivity and exclusivity of the assay were evaluated using 6 strains belonging to 3 species. The standard strains of *Bacillus cereus* were used – *Bacillus cereus*, obtained from the Ukrainian Center for Control and Monitoring of the Ministry of Health of Ukraine, *Bacillus cereus* from LGS Standards the type collection of microorganisms ATCC 11778, *Bacillus cereus* from Ukrainian collection of microorganisms (UCM) B 5650 and B 5671. As a control, *Paenibacillus polymyxa* B 5760^T *Paenibacillus macerans* B 5803^T were used.

Fresh green peas, lettuce, canned green peas, apple juice and dried herbs have been used for the studies. We studied the washings of fresh vegetables and dried herbs, bay and the juice from canned food. All the canned food samples were previously confirmed negative for *B. cereus* by plate count. The samples were inoculated with overnight culture of each strain ($\sim 10^8$ CFU) in the ratio 1:1. Further research was carried out in two variants: 1) 1,5 ml of liquid inoculated with bacteria was taken for DNA extraction without any pretreatment; 2) 2,5 ml of liquid inoculated with bacteria suspension was centrifuged 1 min at 2000 rpm to remove organic substances. The samples were filtered through nitrocellulose membrane filters «Millipore» (d 0,22 microns), after filtration filters were taken for DNA extraction. The DNA extraction procedure was performed using "Reagent kit for the isolation of genomic DNA from bacterial cultures". DNA samples were stored at -20 °C.

The second preliminary sample processing protocol for the detection of *B. cereus* was the best for the analysis of toxin-producing *Bacillus cereus*, based on PCR.