Safety and toxin-producing ability of bacillary microbiota of Ukrainian plant raw materials and products.

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Short Communication

The distinction and identification of bacilli as the representatives of the plant raw material epiphytic microbiota is a necessary step for forecasting and ensuring the safety of plant products. It allows promptly adjusting their technological processing, which ensures the accordance of food products to the international standards. Standardized methods of diagnosing the safety of food and raw materials are classical methods of food microbiology, based on the phenotypic characteristics of microorganisms, which are time-taking and not always able to diagnose the microbial toxigenic properties [1]. The analytical information about the inaccuracy of the indication of bacillary food poisoning agents, the need for preventive analysis of risks associated with aerobic and facultative anaerobic spore forming microorganisms of *Bacillales* order results in the urgency of their detection by accelerated modern methods [2].

The purpose of the work was to characterize the composition of the microbiota of plant raw materials and products of their processing and to develop accelerated molecular biological diagnostics of bacillary food poisoning agents and product spoilage (contaminants of the genuses *Bacillus*, *Paenibacillus*) by the genetic determinants of their toxicity. Characteristics of microorganisms were studied with standardized classical methods by phenotypic properties and molecular biological methods [3].

For the first time the group composition of microbial contaminants of 217 samples of different plant raw material species and products of their processing were investigated according to the mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM), mold fungi and yeasts quantity. The dominance of MAFAnM by 2-3 orders among the groups of microorganisms was established [4]. The research of the group composition of microbial contaminants of the most widespread plant raw material species shows that MAFAnM varies from 4.6×10^4 CFU/g on tomatoes surface to 8.4×10^6 on carrots surface, while the quantity of mold fungi varies from 2.7×10^2 CFU/g on pepper to 5.2×10^4 on carrots, the quantity of yeasts from 0.9×10^2 on vegetable marrows to 1.7×10^5 CFU/g with carrots. The biodiversity of microbiota vegetating on tested plant raw material was mainly formed by the spore-forming bacteria species that are the potential agents contaminating of the foodstuff [5,6].

By phenotypic characteristics of contaminants from 217 food samples, which were studied by standardized classical methods; it was found that they belong to 9 morphotypes. Among the bacillary contaminants of the samples, the *Bacillus subtilis* and *Bacillus licheniformis* group is the most numerous one (20 to 37% of total bacilli count), *Bacillus megaterium* was detected in amount of 6 to 21%, *Bacillus pumilus* in amount of 4 to 13%, *Bacillus circulans* in 2 to 7%, gas-forming *Paenibacillus polymyxa* and *Paenibacillus macerans* (the causative agents of bombarding spoilage) are in amount of 3 to 14% and 2 to 9% respectively, while the microorganisms of the *Bacillus cereus* group (in particular *Bacillus cereus* and *Bacillus thuringiensis*) are in 10-31% and 4-13% respectively. Analysis of potential pathogens of food intoxications, toxicoinfections and food spoilages occurring among the biodiversity of microbiota vegetating on the investigated samples, which consist mainly of bacteria with predominance of spore-forming species, was shown to be complicated and long time-taking [7,8].

The priority technique for preliminary preparing food samples has been developed by us and Polymerase Chain Reaction (PCR) parameters with selected group and species specific primers have been optimized. PCR was carried out with specific primers to detect toxin codes in various kinds of bacilli genes: *nhe*, *hbl*, *cytK*. Molecular genetic diagnosis showed the specificity of the contaminants in Ukraine, the presence of the *nhe* gene was detected in 100% of *Bacillus cereus* strains, *hbl* in about 60% and *cytK* in about 40% of the strains studied. This provides a way to diagnose regulated bacillary contaminants in the test samples that affect the safety of products by *Bacillus cereus* strains. It should be noted that the presence of the toxicity gene was found in a typical saprophytic strain. Studies of food raw materials and products have confirmed the need to improve microbiological control of product safety by introducing accelerated specific diagnostics of contaminants by molecular genetic methods.

Conclusion

As a result of the research, a microbiota composition of plant raw materials and products of their processing was studied; accelerated molecular biological diagnosis of bacillary food poisoning agents and product spoilage was elaborated. A technique for preliminary preparation of food samples was developed and the Polymerase Chain Reaction parameters were optimized with selected group and species-specific primers for diagnosing regulated bacillary contaminants that affect

the products' safety in the tested samples. Such diagnostics will allow producing new competitive products of guaranteed quality and microbiological safety.

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