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Identification of the Clostridium perfringens by a single-copy of the COL(A) gene (#426)

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Introduction

The production of sterilized canned meat products involves the use, in addition to meat and vegetable raw materials, spices. Knowing that of them there is a real danger to the quality of canned food, and the consumer is crucial .

There are in spices Salmonella spp., E. coli, toxigenic molds, incl. various species of Aspergillus, as well as spore microorganisms of soil origin - B. cereus and C. perfringens.

One of these microbial contaminants, *C. perfringens*, has spores with high thermal stability, which during heat treatment of the canning mass and tightness of the packaging are not only not inactivated, but rather receive stimuli and conditions for vital activity.

Only express detection of the required spore-forming microorganisms would allow taking corrective measures to prevent the microbiological contamination of canned food.

For example, PCR (polymerase chain reaction) with oligonucleotide primer recognition of the alpha toxin of the *C. perfringens* gene underlay the creation of the Clostridium perfringens Detection Kit Biotecon Diagnostics SL express system developed for the detection of *C. perfringens* in food products in environmental objects and in clinical material.

In samples of chicken carcasses purchased in the distribution network, multiplex PCR, turkish scientists identified and typed *C. perfringens* by detecting the toxin genes in the cpa, cpb, etx, iA, cpe and cpb2 genomes .

The aim of this work was to identify *C. perfringens* in samples of bay leaves used in the production of sterilized canned meat using PCR using primers developed by us.

For PCR diagnostics of *C. perfringens*, species-specific primers for the collagenase target gene were used

(BAB79879.1 – NCBI protein ID) .

Methods

Bay leaf samples, *C. perfringens* ATCC 13124 strain, colA gene collagenase *C. perfringens* ATCC 13124, C. sporogenes 284, oligonucleotide primers: forward primer to the cola-TGGGTAAGTTTAGAGAAGACCCA; reverse primer to the gene colA – ATTGAGCCTTAACTTCCTTTGAA.

The protocol was adapted to identify *C. perfringens* spores. For this, tenfold dilutions of bay leaf in the buffered peptone water (Oxoid, United Kingdom) were added to the clostridyl broth (DRCM) (Merck, Germany) and heated at a temperature of 80 ° C for 20 minutes.

Then layered on top of the seedlings of vaseline oil and incubated for 72 hours at 37 ° C. For the DNA extraction, a 1.0 mL culture liquid was taken. In the presence of growth in DRCM broth, which was manifested by blackening of the medium in the presence of sulfite-reducing clostridia, a 1.0 mL aliquot was taken from the first dilution and sown on chromogenic clostridial agar (CP Chromoselect) (Sigma-Aldrich, Germany).

Incubation on this dense selective medium was carried out under anaerobic conditions using Anaerocult A (Merck, Germany) for 24 hours at 37 ° C. The growth of *C. perfringens* on this medium is characterized by the presence of green colonies. In the presence of such colonies, they were selected and subcultured on non-selective nutrient agar to obtain a pure culture, which was then confirmed using the API 20A test system (Biomerieux, France).

In parallel with the microbiology, PCR studies were performed. This is a 1.0 mL aliquot was taken from the proculated DRCM broth, centrifuged for 10 min at 13.4 thousand revolutions. A precipitate of DNA reagents was used (Syntol, Russia). The PCR was performed on ANK 32 instrument (Syntol, Russia).

Results

For screening *C. perfringens*, naturally contaminated samples were used, namely 19 bay leaf samples. Confirmation of the results obtained by real-time PCR was performed using the classical microbiological method. Simultaneously with the test samples, the controls were investigated: as a positive control, the strain *C. perfringens* ATCC 13124; as a negative control, the strain of *C. sporogenes* 284.

Of all the samples studied bay leaf microbiological method *C. perfringens* was detected in four samples, PCR method - in five samples. The discrepancy between the results obtained by PCR analysis and the microbiological method for one of the samples may presumably be due to the initially low content of C. perfringens spores, which turned out to be beyond the sensitivity range of the classical microbiological method. The detection of *C. perfringens* in bay leaf samples by the PCR method made it possible to increase the detection of this microorganism due to the sensitivity of the method, while reducing the duration of the analysis to 48 hours.

Conclusion

The developed PCR method already at the stage of enrichment of the object under study on nutrient media can be used for the accelerated determination of *C.perfringens*. However, *Clostridium spp* is of no small importance in ensuring the quality and safety of food products. Therefore, the development of primers to identify other members of the genus *Clostridium* is relevant, which is possible using the gene-collagenase as a marker.

Notes