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Applying MPN (Most Probable Number) Method Combined with PCR (Polymerase Chain Reaction) Procedure for Enumeration of *Clostridium botulinum* Spores in Honey Samples

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Abstract

Scientific studies have recognised the great medicinal value of honey. However, *Clostridium botulinum* spores have been found in honey that was implicated in infant botulism, a neuroparalytic disease caused by a neurotoxin produced in the infant's intestine after spores of these bacteria are ingested and grow.

The objectives of the present study were to produce spore suspensions of different strains of *C. botulinum*, A, B, C, D, E, and F and to enumerate spores of these strains in spiked honey samples by using an enrichment followed by a PCR procedure, namely MPN-PCR (Most Probable Number - Polymerase Chain Reaction) method, targeting the neurotoxin genes.

Spores of different strains of *C. botulinum* were produced by applying various media. During the anaerobic incubation at 30–35 °C for 4–7 d, the sporulation degree was checked daily with a phase contrast microscope. The cultures that resulted in a very good level of sporulation, more than 60 % of vegetative cells sporulated, were harvested by centrifugation at 500 g, washed 3 times with sterile ice-cold water, and re-suspended in phosphate buffer saline (PBS). The spore suspensions were stored at 4 °C.

Sterilized honey samples were spiked with a known amount of spores of different strains of *C. botulinum*, with each strain separately and a mixture of all the strains. The enumeration of spores was carried out by applying MPN-PCR method. Two media, FAB and CMM, were used as enrichment media. The number of individual strain and mixed strain spores in the spiked samples were similar to the number of spiked spores. The results were similar between the two enrichment media. It can be concluded that the applied method can be used to enumerate *C. botulinum* spores in honey samples despite of the high concentration of sugar in honey.

Keywords: *Clostridium botulinum*, enumeration, honey, infant botulism, MPN-PCR, spores