Toxin Production by Clostridium botulinum type A- Use of Bioassay

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Abstract

Clostridium botulinum is one of the important food borne pathogen related to food safety. It comprises of six groups of bacteria and produce a family of seven distinct protein neurotoxin types (A through G) referred to as botulinum neurotoxins (BoNTs). BoNTs are antigenically distinct but produce similar effects on host. These are the most powerful toxins known to mankind and responsible for number of outbreaks of foodborne botulism in human beings throughout the world. The endopeptidase activity assay has been adopted to provide a specific measure of BoNT/A activity in culture supernatants of proteolytic C. botulinum type A. The minimum detection limit was 0.2 MLD 50 /ml indicating the assay as more sensitive than the standard mouse bioassay or any other in vitro assay available to date. Growth studies at 15°C, 25°C and 37°C with C. botulinum strain NCTC 7272 demonstrated that the first appearance of BoNT/A (0.1-1.0 MLD50/ml) occurred during mid-late exponential or early stationery phase of growth. Extracellular BoNT/A formation were not proportional to the viable count. Slightly more BoNT/A was detected at 25°C than at 37°C or 15°C. The results of BoNT/A formation by one of the growth curves at 25°C measured by endopeptidase assay and the mouse bioassay were similar confirming the specificity of the assay. A simple method was developed to lyse the cells so that BoNT/A formation could be subsequently measured in the endopeptidase assay. The conversion of BoNT/A from the single-chain to dichain form during growth has also been measured.