

Study of neurotoxin production process using *Clostridium botulinum*

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Abstract

Toxin is a product of bacterial metabolism and is excreted by the organism into the surrounding medium during the life of *Clostridium botulinum*. The yield of botulinum neurotoxin type A (BoNT/A) might be dependent on cell mass depending upon the various culture conditions. The influencing factors like inoculum size, pH, NaCl concentration, glucose concentration, and so on, were examined. The optimum culture conditions for *C. botulinum* cell mass were 2% inoculum size, 0.5% NaCl, 0.4% Glucose concentration without pH control in TPM medium. BoNT/A was produced by *C. botulinum* cell lysis. Toxin production related to autolysis of the organism. Old culture could enhance the toxin production in young culture. The optimum lysis induction condition for *C. botulinum* BoNT/A was the addition of 2% (v/v) of 4days of old culture at 1.5th day of young culture. For the purification, the BoNT/A was precipitated from the culture by acidification at strong acid pH, and the precipitate was washed with water at mild acid pH. BoNT/A was extracted from the washed precipitate with 0.075M calcium chloride and precipitated from the extract at strong acid pH. Extracted BoNT/A was dissolved into potassium phosphate buffer at neutral pH. And then, anion exchange chromatography was carried out for the more purification of BoNT/A. BoNT/A was fractionated on Q-sepharose CL-4B column with 20mM NaCl. Most of the information obtainable from the SDS-PAGE analysis of these substances was also obtained by gel filtration on a Superdex-200 column. Eluted BoNT/A fraction was identified the pure botulinum neurotoxin type A for SDS-PAGE analysis.

References

1. P. T. Sinpe and H. Sommer, Studies on botulinus toxin. 3. Acid precipitation of botulinus toxin (1928), *J. Infectious Diseases*, 43, 152-160.
2. Y. Humeau, F. Doussau, N. J. Grant and B. Poulain, How botulinum and tetanus neurotoxin block neurotransmitter release (2000), *Biochimie*, 82, 427-446.