Localization of Lecithinase Activity in Clostridium perfringens

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The lecithinase of Clostridium perfringens is an exoenzyme (3). However, lecithinase activity was demonstrated in cytoplasmic extracts obtained from mechanically disrupted vegetative cells of type A C. perfringens (4). This report deals with the intracellular and extracellular lecithinase activities of vegetative cells and spores of type A C. perfringens.

Strain BP6K (1), which produces considerable quantities of lecithinase, and strain Hobbs 3 (NCTC 8239), which produces small amounts of lecithinase, were used in this study. Both strains were grown for 5 hr at 46 C in 1 liter of medium which consisted of 20 g of Trypticase (BBL), 5 g of yeast extract, 2.5 g of starch, 1 g of sucrose, 1 g of K2HPO4, 0.1 g of MgSO4 - 7H2O, and 0.1 g of Fe2(SO4)3 - nH2O. The medium was adjusted to pH 6.8. Spores were prepared according to the method of Schneider et al. (6).

Vegetative cells and spores were harvested by centrifugation, washed five times with 0.05 M tris(hydroxymethyl)aminomethane (Tris) buffer (pH 7.2), suspended in 0.85% saline, and fractionated (100 mg/ml, wet weight). The vegetative cells were fractionated at 25,000 psi by the use of a Sorvall Ribi Cell Fractionator (model RF-1). Spores were fractionated at 50,000 psi. The efficiency of disruption was estimated by examination of stained preparations of the fractionated material; disruption of the vegetative cells was complete. However, only 60% of the spore preparations were disrupted. The fractionated material was separated into soluble and particulate fractions by centrifugation at 35,000 X g. Each particulate fraction was washed five times with buffer and stored at 4 C.

The two fractions and the culture filtrates were assayed for lecithinase activity using the lecithoventillin reaction (7). For our method, we prepared a standard assay curve using commercial lecithinase (5). The lecithinase activity of culture filtrates and the fractions were interpolated from the standard assay curve.

The culture filtrate of strain BP6K contained approximately six times more lecithinase than did the culture filtrate of strain Hobbs 3 (Table 1). Intracellular (soluble fraction) lecithinase was present in both strains in comparable quantities. No lecithinase activity was detected in the particulate fraction (membrane fraction).

The soluble fraction of the spores of each strain possessed a lecithinase activity of 18 µg/ml. The particulate fractions of the spores were without activity. The commercial preparation of lecithinase (Nutritional Biochemicals Corp., Cleveland, Ohio) was processed through the fractionator without a loss of enzymatic activity.

These results indicate that strains of C. perfringens that produce large quantities of lecithinase excrete most of their lecithinase into the surrounding medium. On the other hand, strains that produce less lecithinase retain approximately 50% of the enzyme in the cytoplasm. Some of the extracellular lecithinase activity may be due to the lysis of the vegetative cells. A rise in the toxicity of cultures of C. botulinum type B was correlated to the lysis of vegetative cells (2).

It is evident from our studies that the total lecithinase activity of C. perfringens may not be determined if only culture filtrates are assayed for lecithinase activity.

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