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CLOSTRIDIUM PERFRINGENS SPORE-LYTIC ENZYMES(U)
MASSACHUSETTS UNIV AMHERST R LABBE 10 SEP 83
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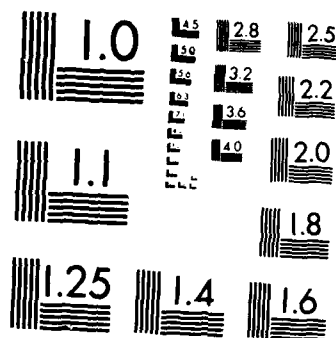
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20. (cont.)

but it could only be obtained by reagents known to disrupt the spore coat. ~~We developed~~ a fluorescence assay for determining the hydrolysis product of these enzymes which will help future work in this area. Results indicated the presence of a third spore-lytic enzyme associated with the wall of the organism. Its role and function is unknown.

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Final Report

1. Participating Scientific Personnel

Dr. Shirley Tang

Dr. Ronald Labbe

Hsiao-ping Lu, received M.S. degree while associated with contract

David Gombas, received Ph.D. degree while associated with contract

2. List of Publications

- a. Labbe, R. and S. Tang, 1983. Conditions for production of initiation protein for germination of Clostridium perfringens spores. Canadian Journal of Microbiology, Vol. 29, pgs. 829-832.
- b. Gombas, D. and R. Labbe, 1985. Purification and properties of spore-lytic enzymes from Clostridium perfringens type A spores. Journal of General Microbiology, Vol. 131, pgs. 1487-1496.
- c. Others submitted not yet published.

3. Brief Statement of the Problem

For many years there has been an active interest in the research community on the mechanism by which bacterial spores germinate. Although the chemical stimuli (e.g. nutrients) which stimulate this process have been known for many years an understanding of the biochemical process has been elusive. While most researchers by far have used aerobic spores (Bacillus) we have chosen an anaerobic model (Clostridium). This is because this (1) genus has been neglected (due to difficulty in obtaining sufficient working amounts) and (2) one species, C. perfringens, has an important role in food-borne illness where large numbers of people are served a common meal as one encounters in military feeding situations. We have been working with this species.

The structure of the spore backbone requires that a lytic enzyme be activated during germination in order to allow the core protoplast to

be released and resume vegetative cell growth. The question we sought to answer is what role do spore-lytic enzymes play in the germination process of this organism. What is the nature of such enzymes, their location, properties, modes of action, method of activation, etc. From our previous work we were aware of the existence of two such enzymes from C. perfringens which could cause the germination of its own spores. One was excreted by the vegetative cell (Initiation Protein), the other associated with the spore itself (spore-associated spore-lytic enzyme).

4. Summary of Results

a. Spore-Associated Spore-lytic Enzyme

We found that the spores C. perfringens contain at least two spore-lytic enzymes which are active in hydrolyzing cortical peptidoglycan. One enzyme was purified 1800-fold and has a molecular weight of 17,400. Two protein bands were apparent after sodium dodecyl sulfate - polyacrylamide gel electrophoresis. The isolated enzyme was investigated for response to temperature, pH, ionic strength and enzyme inhibitors. A second enzyme differing from the first in apparent molecular weight (29,800) as determined by gel exclusion chromatography, and also in its pH optimum and activity on cortical substrate, was also isolated, although not purified to the same extent. Both enzymes are associated with the coat protein layers.

b. Initiation Protein (IP)

Our main effort was associated IP. We improved the purification procedure by using batch ion exchange chromatography followed by gel permeation chromatography. This was the method of hydroxylapatite chromatography. These procedures resulted in a 1700-fold purification with a 47% recovery of IP. Binding studies with streptolysin A indicated

compounds of different hexoses we have shown that IP is not a N-acetylmuramidase (lysozyme) or an N-acetylglucosaminidase. The IP released free amino groups from its substrate (cortical fragments). Further analyses indicated that this was due to the release of N-terminal alanine. The IP is therefore an amidase. In order to determine the molecular weight of the hydrolysis product of IP we developed a procedure of fluorescent-labeling of the cortex. After IP action soluble fluorescent products are measured using a fluorometer. Near the end of the contract period efforts were underway to measure the size of the fluorescent reaction products.

c. Other Spore-Lytic Enzyme

In addition to the spore-lytic enzymes mentioned above we have found an additional intracellular one. Our results indicate it is weakly bound to the cell wall since neither detergent nor high salt showed significant increase in enzyme yield over low salt buffer extraction. The enzyme was partially purified and has an apparent molecular weight of 100,000. Included in our future efforts will be attempts to characterize this enzyme. At present its role is unknown.

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