STUDY OF HEAT RESISTANCE IN A BACTERIAL NUCLEOSIDASE

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ABSTRACT

The enzyme nucleosidase(EC.3.2.2.1) is present in the intact spores, germinated spores as well as vegetative cells of Bacillus cereus T. In the intact spores the enzyme is resistant to heat and, in fact, has a high temperature optimum. Though the spores themselves become sensitive to heat on germination, the enzyme retains its resistance to heat on germination as well as its high temperature optimum. The vegetative cell enzyme is sensitive to heat. The enzyme in all types of cells & spores is resistant to octyl alcohol. There is a close correlation between the development of heat resistance in the sporulating cells and that of heat resistance of the enzyme.

INTRODUCTION

Recently keen interest has been taken in the biochemical changes taking place during sporulation in bacteria (1,2,3,4).It has been shown that the spore contains a few active enzymes (3,5,6,7,8).It has been observed that during germination of spores of Bacillus cereus T in the presence of L - alanine and a purine riboside, there is always a cleavage of the purine riboside (6,7). There are reports to indicate that this cleavage of the riboside can be both hydrolytic (9,10,11) as well as phosphorolytic(12,13). Hence, there is an indication that two different types of nucleosidase (EC.3.2.2.1) occur in nature.

There are several reports of heat stable enzymes in spores, such as alanine racemase (14),nucleosidase (9,10), catalase (11) etc. In view of these, it is possible that the heat stability of the spore enzymes associated with the germination process may have some common relation.

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ship with the resistance of bacterial spores to heat & deleterious agents. Nucleosidase (EC.3.2.2.1) is one such enzyme. Hence, a careful study of the heat resistance characteristics of nucleosidase (EC.3.2.2.1) present in Bacillus cereus T cells and spores, & correlation, if any, in the occurrence of heat resistance in this enzyme & in the sporulating cells was considered desirable. This report describes such a study.

MATERIAL AND METHODS

Culture Conditions: The organism, Bacillus cereus T, was obtained from United States Department of Agriculture, Washington, D.C.and subsequently maintained at this laboratory on nutrient agar slants. The organism was grown in the "Glucose-Yeast Extract-Minerals Medium" (G Medium) (3) previously autoclaved at 15 lbs. pressure for 20 min. The vegetative cells & spores were prepared by growing the organism in G - Medium by the " active culture technique "(2,15). All cultures were grown by incubating at 30±1 C on a rotary shaker (speed 160 rpm).The intact spores were ready for harvest after 30 hours.

Measurement of pH,Growth & Cell Counts: The pH of the culture was determined using a Toshniwal (India) pH meter. Growth was measured with the help of Klett Summerson Photoelectric Colorimeter using blue filter. A close check on the morphological state of the cultures was kept by microscopic examination of the stained smears. Total viable cell count and heat stable cell count (at 80 C for 30 min.) were determined by plating suitable dilutions on solid nutrient agar medium & incubating at 30±1 C for 24 hours.

Assay of Nucleosidase (EC.3.2.2.1) Activity:The cells & spores were harvested by centrifuging at 8000 rpm for 10 min. at 0 - 4 C using Servall's Refrigerated Superspeed Centrifuge Model RC-2. Then they were washed twice with 20ml-aliqotes of cold,0.2M phosphate buffer (pH 7),suspended in 10ml buffer & finally incubated at the required temperature with adenosine. The enzyme activity was assayed

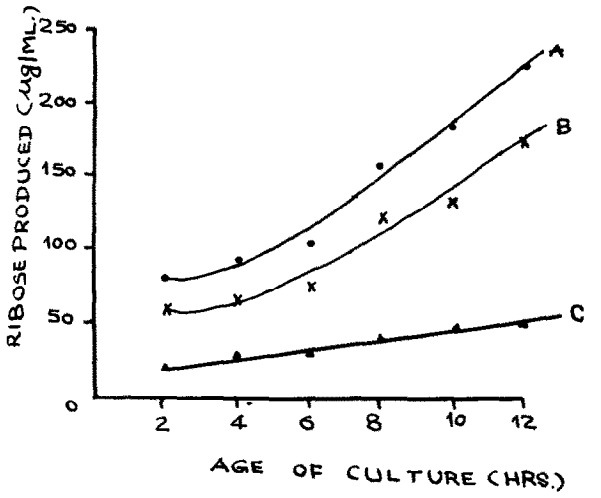

Fig.1.: Nucleosidase (EC.3.2.2.1) activity in the vegetative cells (20µg/ml)of different culture age, incubated with 600 µg adenosine at 37 C for 20 min.Symbols: A, enzyme activity in the ~~xxxxx~~ Octyl Alcohol Killed-vegetative cells; B, enzyme activity in the normal vegetative cells; C, amount of ribose utilized by the normal vegetative cells (C=A-B).

in terms of ribose liberated using Nelson's method of determining the reducing sugars of blood (16,17,18).

Materials used: Adenosine was obtained from Zellstofffabrik Waldhof,W. Germany.D(-)Ribose was procured from British Drug House (India) and yeast extract from A.Costantio & Co.,Italy.A.R.grade chemicals were used in all the experiments.

RESULTS AND DISCUSSION

The present studies indicate the presence of a nucleosidase(EC.3.2.2.1) in the vegetative cells & spores of Bacillus cereus T, which is hydrolytic in its action on adenosine since significant amounts of ribose are liberated into the medium on incubation of cells/spores with adenosine.

Nucleosidase(EC.3.2.2.1) Activity in the Normal, & Octyl Alcohol-Killed Vegetative Cells: The activity was measured at 37 C in the normal vegetative cells of different culture age, and also in the corresponding octyl alcohol killed-vegetative cells (Fig.1). The data reveal the presence of considerable enzyme activity at 37 C in both types of vegetative cells. However, the attempts to measure the enzyme activity at 75 C proved futile. No enzyme activity could be detected. Thus, the

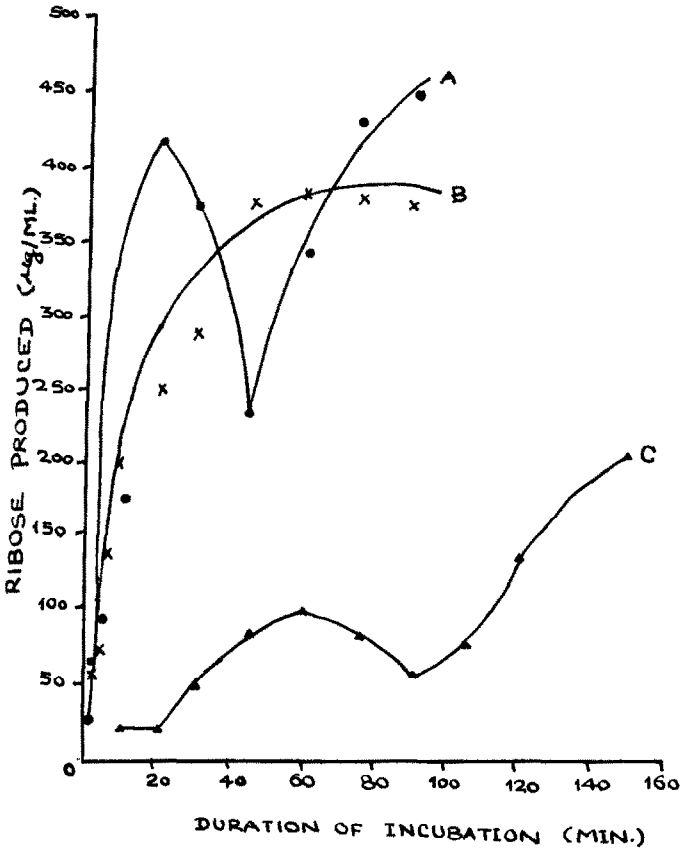

Fig.2.: Nucleosidase (EC.3.2.2.1) activity in the intact spores (100 μg/ml) and in the Octyl Alcohol Killed - Germinated Spores (100 μg/ml) incubated with 1500 μg adenosine. Symbols: A, enzyme activity in the intact spores at 75 C; B, enzyme activity in the octyl alcohol killed-germinated spores at 75 C; C, enzyme activity in the intact spores at 30 C.

vegetative cells contain a nucleosidase(EC.3.2.2.1) species which is sensitive to heat but not to octyl alcohol. This observation is meaningful in view of the differentiation reported between heat resistance and octyl alcohol resistance of the sporulating cells of Bacillus cereus T (19). The enzyme activity, at 37 C, increased consistantly with the age of the vegetative cells, irrespective of the octyl alcohol treatment (Fig.1). The higher level of enzyme activity obtained with the octyl alcohol killed vegetative cells is due to the possibility that cells killed by octyl alcohol are unable to further metabolize ribose liberated. Normal cells might utilize part of the ribose liberated by the activity of the enzyme on adenosine. It is apparent from Fig.1 that this extent of ribose utilization corresponds to about one-fourth of the ribose liberated

by the enzyme present in the octyl alcohol killed-vegetative cells. It is also apparent that the ribose utilization increases linearly with the age of the vegetative cells.

Nucleosidase(EC.3.2.2.1) Activity in the Intact Spores , And in the Octyl Alcohol Killed-Germinated Spores: In the intact spores , the enzyme activity was assayed both at 30 C and 75 C. The data obtained at both the temperatures (Fig.2) indicated a linear increase in the ribose production with the duration of incubation during the initial stages. There was, however, a break in the curve after 20 min. of incubation at 75 C. In case of enzyme activity curve at 30 C, the break was observed after 60 min. of incubation. These breaks in the enzyme activity curves could conceivably be due to further utilization of the ribose liberated because in case of octyl alcohol killed-germinated spores no such sharp break in the enzyme activity curve was noticeable (Fig.2). It was also observed that the enzyme activity in the intact spores was higher at 75 C indicating that the enzyme was resistant to heat and had a high temperature optimum.

In the octyl alcohol killed-germinated spores the enzyme was stable to heat and the activity at 75 C was comparable to that in the intact spores (Fig.2).

Occurrence of Heat Resistance in the Nucleosidase(EC.3.2.2.1) And Sporulating Cells: The data on the occurrence of heat resistance in the enzyme and in the sporulating cells grown at 30 C indicated that acquisition of heat resistance by this enzyme and the formation of heat stable spores occurred simultaneously (Table 1). A slow and constant increase in the ribose liberation by the enzyme incubated at 75 C was observed from 8th to 12th hour of culture age but at 13th hour an abrupt increase in the ribose liberation was observed. This abrupt increase in the enzyme activity at the culture age of 13 hours inspite of the high temperature of incubation (75 C) reflects upon a probable occurrence of heat resistance in the enzyme at that time. A study of the heat stable cell count showed them to be low at 11 and 12 hour culture age but at 13th hour (pH 7.4)these were much higher . It is apparent from Table 1 that the heat stable cells have increased from 10% at 12 hour to 70% at 13 hour culture age.

It would be noted from Table 1 that at 14th hour culture age the heat stable cell counts are higher than the respective total viable cell counts. This is because of the fact that on giving heat treatment (at 80 C, for

Table 1. : Occurrence of Heat Resistance in the Nucleosidase (EC.3.2.2.1) and Sporulating Cells grown at 30 C.

Age of Culture (Hours)	pH	Ribose Produced (µg/ml) by the Enzyme Incubated at 75 C for 10 min.	20 min.	30 min.	40 min.	Total Viable Cells/ml	Heat Stable Cells/ml	Stain
8	7.10	195	215	235	280	2.6×10^8	Nil	Cells granulated, taking stain, forming clumps.
9	7.15	210	230	250	305	2.1×10^8	Nil	-do-
10	7.20	220	250	280	335	2.2×10^8	Nil	-do-
11	7.25	235	280	320	390	1.7×10^8	3.5×10^6	Spore shape formed, stainability reduced.
12	7.35	255	295	395	465	2.0×10^8	2.1×10^7	-do-
13	7.40	305	335	525	595	1.6×10^8	1.1×10^8	Refractile spores, in clumps & chains.
14	7.55	440	515	565	630	1.8×10^8	2.9×10^8	-do-

30 min.)clumps & chains of the sporulating cells are broken into individual cells resulting into heat stable cell counts higher than total viable cell counts.

The results indicate that the nucleosidase (EC.3.2.2.1) present does not lose its heat resistance during germination of the spores of Bacillus cereus T. The vegetative cells of the organism synthesize the heat - sensitive, octyl alcohol - resistant form of the enzyme which becomes resistant to heat at the same time as the cells develop heat resistance. The results are in conformity with the findings of earlier workers (20,21).

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