DEPENDENCE OF THE HEAT RESISTANCE OF BACTERIAL SPORES ON THE CALCIUM: DIPICOLINIC ACID RATIO

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Dipicolinic acid (pyridine 2, 6 dicarboxylic acid) constitutes 5 to 15 per cent of the dry weight of the spores of both aerobic and anaerobic bacteria. [1] The dipicolinic acid (DPA) is excreted when spores germinate (Powell and Strange, 1953). Spores contain 2 to 10 times as much calcium as vegetative cells (Curran et al., 1943).

Evidence regarding the essentiality of DPA to the heat resistance of spores has been conflicting. Ca^{++} has clearly been involved in thermal resistance (Sugiyama, 1951; Slepecky and Foster, 1959). It has been suggested (Young, 1959) that dipicolinate and Ca^{++} could act together in stabilizing essential proteins and nucleic acids. In view of the postulated close associ- (whether as chelate, salt, or other complex) of DPA and Ca^{++} in the spore we believed that there might be a relationship between the Ca:DPA ratio and heat resistance, rather than between either of these substances, considered separately, and heat resistance.

We have determined the heat resistance (ability to form colonies after exposure at 90 C for 20 min) of spores of several species of Bacillus,

[1] Dipicolinic acid has also been reported in the slime of Natto, a Japanese food prepared from steamed soybeans inoculated with Bacillus natto (Udo, 1936), and in culture filtrates of Penicillium (Ooyama et al., 1961).

harvested from a medium containing liver fraction "B" (Wilson and Company, Chicago, Illinois). DPA (method of Janssen et al., 1958) and total Ca^{++} (method of Roe and Kahn, 1929, as adapted by Slepecky and Foster, 1959) of spores and the relationship of these substances to heat resistance have been examined.

There is no apparent direct relationship between either the DPA or Ca^{++} content of these spores and their heat resistance (table 1). Indeed, the most heat resistant organism we have tested, Bacillus subtilis QM B1564, has less dipicolinic acid than the most sensitive organism, B. cereus var. mycoides, QM B1579; spores of B. megaterium, QM B1551, contain more Ca^{++} than those of B. subtilis, yet the latter is the more heat resistant.

Table 1

Calcium, dipicolinic acid, and heat resistance of Bacillus spores

Spores of	Ca^{++} [a]		DPA [a]		Ca:DPA		Survival	
	μg	μmoles	μg	μmoles	molar ratio	rank	%	rank
B. megaterium, QM B1551	28	0.70	95	0.57	1.22	2	0.27	2
B. cereus var. mycoides, QM B1579	16	0.40	79	0.47	.85	4	0.0007	4
B. subtilis, QM B1564	19	0.48	59	0.35	1.42	1	27.8	1
B. cereus, QM B1565	11	0.27	44	0.26	1.04	3	0.002	3

[a] Values indicate amount of Ca^{++} or DPA per mg of spores. DPA (mol. wt. 167.2), purchased from Aldrich Chemical Co., Milwaukee, Wisconsin, was used as standard in analyses.

We have compared the relative ranks of the Ca:DPA ratios for the different bacterial species with the ranks of these species in heat resistance (survival). The highest Ca:DPA ratio is associated with the most resistant organism, and the lowest ratio is associated with the lowest heat resistance.

The other species show good agreement in relative ranks of heat resistance and Ca:DPA ratio.

We have altered the Ca:DPA ratio of B. megaterium spores by growing the organism in liver "B" medium to which increasing concentrations of Ca^{++} (as $CaCl_2$) had been added. Spores with Ca:DPA ratios ranging from 1.22 to 1.85 were obtained, and there was, indeed, increased thermal resistance with increased Ca:DPA ratios (table 2 A). Our results are not comparable with those of Black et al. (1960), who found that when B. cereus strain terminalis formed spores in distilled water ("endotrophically"), DPA accumulation depended on Ca^{++} concentration in the sporulation medium. Heat resistance increased with increasing DPA, but no data on the Ca^{++} content of the spores was given. Our basal liver "B" medium contains approximately 15 mg of Ca^{++} per liter, and, while increasing the concentration of Ca^{++} in the sporulation medium does not appreciably alter the DPA content of the spores, their heat resistance is increased. Unfortunately, we were unable to sporulate our strain of B. megaterium endotrophically so as to effect a significant reduction in spore Ca^{++}, nor were we able to bring about really dramatic changes in concentration of DPA.

Contrary to the observations of Church and Halvorson (1959), we were unable to reduce the DPA concentration of spores by addition of 0.2 per cent phenylalanine to the growth medium. However, growth of B. megaterium on liver "B" medium, to which 0.2 per cent alanine had been added, resulted in the production of spores with increased DPA. Such spores were less heat resistant and had a Ca:DPA ratio which was lower than that of spores grown either in the basal liver "B" medium, or in this medium with added phenylalanine (table 2 B).

Table 2

Effect of alteration of calcium and dipicolinic acid content of Bacillus megaterium spores on their heat resistance.

A. Spores grown on liver "B" with added Ca^{++}.

Medium additive	Ca^{++}		DPA[a]		Ca:DPA	Survival
	μg	μmoles	μg	μmoles	molar ratio	%
0[b]	28	0.70	95	0.57	1.22	0.18
Ca^{++} (0.1 mM)	31	0.77	94	0.56	1.37	0.32
Ca^{++} (0.5 mM)	40	1.0	91	0.54	1.85	2.9
B. Spores grown on liver "B" plus phenylalanine or alanine (0.2%).						
0[b]	28	0.70	94	0.56	1.25	0.35
Phenylalanine	28	0.70	99	0.59	1.18	0.18
Alanine	25	0.63	122	0.73	0.86	0.032

a Values indicate Ca^{++} or DPA per mg of spores. Failure to remove Ca^{++} by washing with N/1 HCl indicates that Ca^{++} is not extraneously absorbed on the spore.

b Small differences in these spores is to be expected since they were grown at different times. However, spores used in each section of this table (A or B) were grown and harvested at the same time.

While the observed agreement between the Ca:DPA ratio and heat resistance of spores is admittedly not a perfect one, we feel that the data are sufficiently suggestive to warrant the fuller investigation which is currently under way in our laboratory.

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