THE EFFECT OF GLUCONATE IN PROMOTING SPORULATION IN BACILLUS CEREUS[1]

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Sporulation in the Bacillaceae is a process of unicellular morphogenesis which results in the formation of dormant, heat resistant cell forms. Little is known of the control of sporulation or of the mechanism(s) of dormancy or heat resistance. However, dipicolinic acid (2,6-dicarboxypyridine, DPA) occurs in sporulating Bacillus or Clostridium species concomitantly with heat resistance (Collier and Nakata, 1958; Day and Costilow, 1964). Glucose dehydrogenase is a spore-specific protein which is synthesized early in the sporulation of Bacillus cereus (Bach and Sadoff, 1962). While studying the role of this enzyme in sporulation, it was noted that cultures grown in media containing sodium gluconate formed refractile spores sooner than did the controls. The sporulating cells in gluconate contained DPA, were heat labile, but acquired heat stability at precisely the same time that refractile, heat resistant spores were formed in the control cultures. The experiments described in this communication show that the incorporation of gluconate in sporulation media results in a) reduction of the time required for the initiation of synthesis of morphologically recognizable spores; b) a broad separation of spore morphogenesis from heat resistance and c) a separation of the time of appearance of DPA from the occurrence of heat resistance.

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EXPERIMENTAL

These studies were conducted with B. cereus grown aerobically at 30 C in 3 liter volumes of modified G-medium (Hashimoto et al, 1960) in New Brunswick fermentors. The cultures were stirred at 450 rpm and aerated with 5 liters of air per min. An active inoculum was prepared by successive transfers of the culture in shake flasks of G-medium (Collier, 1957). Identical inoculums were used for normal G-medium and G-medium plus 0.2 per cent sodium gluconate. The 3 liter cultures were sampled at various times during growth and sporulation to ascertain the dry weight of cells/ml, DPA content/ml (Janssen et al, 1958), refractile spores, and heat resistant spores. It should be pointed out that the compound ultimately assayed as DPA may exist in some other functional form in spores. The refractile or phase bright spores/ml were determined by direct counts in a Petroff-Hauser chamber using a phase contrast microscope. Refractile spores could not be stained with crystal violet. Heat resistant spores/ml were determined by plating on nutrient agar after heating suspensions at 80 C for 10 min.

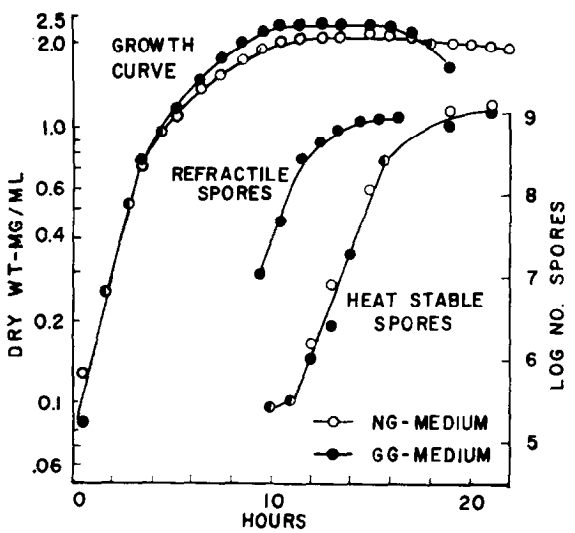

Figure 1. The time course for the growth and sporulation of cultures of B. cereus in normal G-medium (NG) and G-medium plus 0.2 per cent sodium gluconate (GG). Refractile spores are those which appear bright under the phase contrast microscope.

RESULTS

Figure 1 shows the growth curve for B. cereus in the two media used. The presence of gluconate did not affect the exponential growth of the organism. Refractile, non-staining spore structures became apparent microscopically in cultures grown in gluconate medium at 9.5 hours, and they increased exponentially in number from 10^7/ml to a final count of approximately 10^9/ml. Refractile bodies in the control medium first appeared at 13 hours, and the plot of their increase was identical to the curve shown for the increase in number of heat resistant spores. The time course for the development of heat resistant spores in gluconate and control media were identical.

Figure 2 presents the time course for the synthesis of DPA and for formation of refractile spores in cultures grown in gluconate and control media. The presence of gluconate decreases the incubation time required for appearance of these by about four hours.

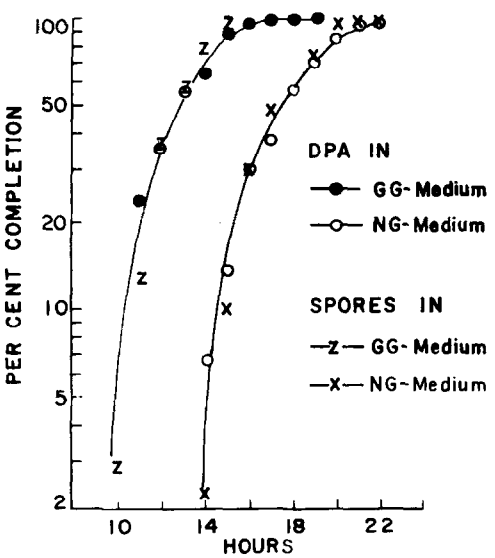

Figure 2. Correlation between the time of synthesis of DPA and the appearance of refractile spores. Heat resistance, refractility and DPA synthesis occur simultaneously in spores produced in normal G-medium (NG).

DISCUSSION

The incorporation of 0.2 per cent sodium gluconate into G-medium containing 0.4 per cent glucose does not affect the exponential growth rate of B. cereus nor the final yield of heat resistant spores. These data suggest that gluconate is not used as an energy source during vegetative growth. Gluconate may function during sporulation in some control capacity, as a key intermediate, or as an energy source. Enzymes for its oxidation to pyruvate have been demonstrated in sporulating cells of B. cereus by Halvorson and Church (1957) and Doi et al (1959); and Nakata and Halvorson (1960) and Hanson et al (1963) have shown that the oxidation of acetate and pyruvate provides energy for spore synthesis.

In sporulating cultures grown in normal medium, the synthesis of DPA, the appearance of refractile spores, and heat resistance occur within a very short time interval of each other. These results differ from those reported by Hashimoto et al (1960) where DPA synthesis and refractility preceded thermal stability by 1.5 hours. The differences might be attributed to a variation in cultural conditions, particularly the higher aeration rate used in the current study.

Refractile spores occurred four hours earlier in cultures grown in gluconate medium than control medium but their respective rates of synthesis were the same. The principal effect of gluconate was to shorten the time interval between the end of exponential growth and the appearance of spore structures. The final DPA content of spores from gluconate and control media were equal, 85 μg/mg dry spores.

The very close correspondence between the percentage completion of refractility and DPA synthesis in gluconate media, shown in Figure 2, leads to the conclusion that each refractile spore contains its full complement of DPA. That is, DPA synthesis per spore is complete four hours preceding heat resistance in that spore. Furthermore, sporulation of the culture is 70 per cent complete in the gluconate medium before it begins in the control

medium. This very broad separation of the time of the occurrence of DPA and heat stability has not been observed previously in cultures of Bacillus species. Halvorson (1957) has shown a separation of DPA synthesis and heat resistance in an anaerobic spore former.

The over-all experimental results indicate that gluconate functions in the initial stages of sporulation but has little effect later in the process. Therefore, cultures grown in the gluconate medium should be useful in studying the sequence of events in sporulation, particularly in the time interval between the occurrence of refractile spores and mature, heat stable spores.

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