ROLE OF TRICARBOXYLIC ACID CYCLE IN BACTERIAL SPORULATION

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Summary α-Picolinic acid, an inhibitor of aconitase, inhibits spore formation in Bacillus megaterium 753, when the cells are grown in medium containing glucose, but it does not inhibits spore formation when the cells are grown in medium containing glutamate, as sole carbon source. The results indicate that the entire tricarboxylic acid cycle may not be necessary for spore formation, atleast in this organism and probably in other spore forming aerobic bacilli.

Tricarboxylic acid cycle (TCA cycle) is believed to be indispensable for sporulation in spore forming aerobic bacilli, since fluoroacetic acid, α-picolinic acid (APA), the latter an inhibitor of aconitase (1) are reported to inhibit spore formation in bacteria (2,3). The results presented in this communication indicate that the presence of entire TCA cycle may not be an obligatory requirement for the process of spore formation in Bacillus megaterium 753.

The cells were grown in shake cultures at 30°C by the active culture technique (4). The growth medium used, contained the following components (in millimolar final concentration): ferric chloride 0.0036, magnesium chloride 0.04, manganese chloride 0.1, sodium sulfate 0.48, calcium chloride 0.75, potassium dihydrogen phosphate 10 and ammonium glutamate 10. The pH of the medium was 6.1. This medium is referred as "glutamate medium". In some of the experiments 10 mM (final concentration) glucose was added to the glutamate medium and the latter medium is referred as "glucose medium". Total viable counts (TVC) were determined by plating appropriate dilutions of the cultures in solid nutrient agar medium.

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The extent of spore formation was determined by treating the cultures with octanol for 15 minutes followed by plating. This treatment kills the vegetative cells and the germinated spores but not the spores. The cells which resist octanol treatment are referred as "octanol stable counts" (OSC) and these represent a mixture of heat labile and heat stable spores. To distinguish between the two types of spores, cultures were heated at 80°C for 30 minutes and then plated. The cells which survive the heat treatment are referred as "heat stable spores" (HSS). The difference between OSC and HSS represents the number of "heat labile spores" (HLS). It may be pointed out here,that one can not distinguish HLS from HSS by microscopic examination or by staining methods. Both the types of spore are similar in shape and refractivility.

The results obtained are summarised in Table one. APA does not inhibits spore formation at 1.5×10^{-3} M concentration, when the cells are grown in "glutamate medium". Whereas APA inhibits spore formation even at lesser concentrations (80% at 10^{-3} M and 100% at 1.5×10^{-3} M), when the cells are grown in "glucose medium".

When the cells are grown on glucose, the energy for the cellular processes is obtained by the oxidation of glucose via glycolysis and TCA cycle. Thus the presence of an inhibitor of aconitase, like APA, would eventually block glucose oxidation as well as the cellular processes like sporulation etc., which depend on the energy supplied by TCA cycle. The results (table 1) are in agreement with this view. It may be noted that TCA cycle is considered essential only for sporulation and not for the vegetative growth (2,3). On the other hand when the cells are grown on glutamate as sole carbon source, aconitase is bypassed, and now even if the inhibitors of aconitase are present in the growth medium, these would not interfere with the oxidation of glutamate. It is easy to visualize that glutamate can serve as precursor for various cellular constituents as well as it can supply energy by its oxidation through TCA cycle,without the involvement of aconitase.

If complete TCA cycle is not required for sporulation, inhibition of this pathway at non-essential points will not interfere with spore formation. Since APA does not inhibits spore formation, when B. megaterium 753 cells are grown in the "glutamate medium" (table 1), it implies that the enzyme aconitase may not be

Table I

Effect of α-picolinic acid (APA) on growth and sporulation of *Bacillus megaterium* 753

Growth medium used	APA x 10^{-3} M	Cell counts/ml x 10^6			
		16 hours	44 hours		
		TVC	TVC	HLS	HSS
Glutamate	Nil	1.6	8.0	6.0	Below 10^5
Glutamate	1.5	1.56	7.1	6.0	Below 10^5
Glucose	Nil	18.0	94.0	80.0	Below 10^6
Glucose	1.0	17.1	13.0	15.0	Below 10^6
Glucose	1.5	18.3	Below 10^6	Below 10^6	Below 10^6

The vegetative growth is completed by 13 to 16 hours and the sporulation by 38 to 44 hours in this organism. The vegetative cells are long chain like structures. On maturation, each chain gives rise to about 5 spores, due to which TVC is higher at 44 hours as compared to at 16 hours. If vegetative cells are not converted (e.g. in glucose medium in the presence of APA) to spores, they loose their viability after about 25 hours. APA does not inhibits vegetative growth, since TVC are similar at 16 hours, whether the cells are grown in the presence or in the absence of APA. Glutamate seems to repress formation of heat stable spores, because if glutamate is replaced by glucose (as sole carbon source) and inorganic nitrogen in the growth medium, all the spores formed are heat stable (unpublished observation).

involved in the biochemical processes responsible for sporulation and thereby indicating that the entire TCA cycle may not be essential for spore formation in B. *megaterium* 753 and probably in other spore forming aerobic bacilli also.

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