Citrate Formation in B. cereus strain T*

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Nakata and Halvorson (1960) have shown that B. cereus strain T accumulates acetate when grown by the synchronous culture technique of Halvorson (1957) in a medium containing glucose. The accumulated acetate is metabolized during the early stages of sporulation, presumably via the TCA cycle and/or the glyoxalate shunt. The addition of α-picolinic acid during acetate accumulation prevents acetate utilization and specifically inhibits sporulation, but has no effect if it is added after acetate utilization has begun as indicated by the rise in pH of the medium (Gollakota and Halvorson 1960). The latter workers suggested that enzyme systems responsible for acetate utilization are adaptively formed and are required for sporulation. Examination of cell extracts for acetate activation and utilization enzymes led us to observe a system forming citrate from acetate and oxalacetate which is seemingly absent in the cells during vegetative growth but is "induced" when glucose is exhausted from the medium.

Cells of Bacillus cereus, strain T, were suspended in 0.02M potassium phosphate buffer containing 10^{-3}M sodium thioglycollate and broken by two passages through a French pressure cell. The crushed cell preparations, and extracts prepared from them by centrifugation at 15,000 g for 15 min., were tested for their ability to convert acetate and oxalacetate to citrate. Citrate was measured in aliquots of the deproteinized reaction mixture by the procedure of Natelson et al (1948), as modified by Taylor (1953), after extensive evaporation. Acetyl phosphate was determined according to Lipmann and Tuttle (1945). Acetokinase was assayed by the procedure of Rose et al (1954), as modified by Krask (1960). The reaction was stopped with 0.35 ml. of 2M hydroxylamine hydrochloride, adjusted to pH 5.0 with 4.0M NaOH; after 5 minutes the color was developed with the ferric chloride reagent. Table I shows the formation of citrate from acetate and oxalacetate by extracts as a function of age of the culture.

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TABLE I

Age of Culture and Citrate Formation by Strain T, Extracts

Time of Harvesting Cells for Extract	Acetyl Phosphate Found	Citrate Formed
	uM at 60 min.	uM/60 min.
1.5 hr.	8.0	0.1
3.5 hr.	1.1	14.0

Reaction mixture: 2ml: 0.05M tris HCl buffer pH 7.6, 0.3M K-acetate, 0.02M OAA, 0.01M ATP, 0.01M $MgCl_2$, 0.01M cysteine HCl, and extract $\simeq$ 10 mg protein. Incubated at 29°C for 60 min.

Citrate formation, in crushed cell preparations, depends on the presence of acetate, oxalacetate, ATP, $MgCl_2$, and partially on coenzyme A. See Table II.

TABLE II

Requirements for Citrate Formation in Extracts from 3.5 hour Cells.

Component Omitted	uM	Acetyl Phosphate Found uM at 60 min.	Citrate Formed uM at 60 min.
None		1.1	14.0
Acetate	600	1.1	1.6
ATP	20	1.1	3.4
OAA	40	6.5	0.0
CoA	1	2.0	7.4
$MgCl_2$	20	1.6	3.8
Extract	10 mg protein	0.0	0.0

Reaction mixture: Same as shown in Table I.

Conversion of acetate to citrate occurs in extracts of these sporulating cells, but apparently is either nonfunctional or absent from the vegetative cells. Acetyl phosphate accumulation, on the other hand, occurs in extracts devoid of the citrate forming system or of oxalacetate. The acetokinase activities of early and late extracts were found to be essentially the same (1.5 and 1.6 uM acetate to acetyl phosphate mg protein in 15 min. respectively).

The citrate forming activity of the cell free extracts harvested at various times from G. medium[1] is shown in figure 1. The pH of the medium illustrates the time course of acetate accumulation and utilization.

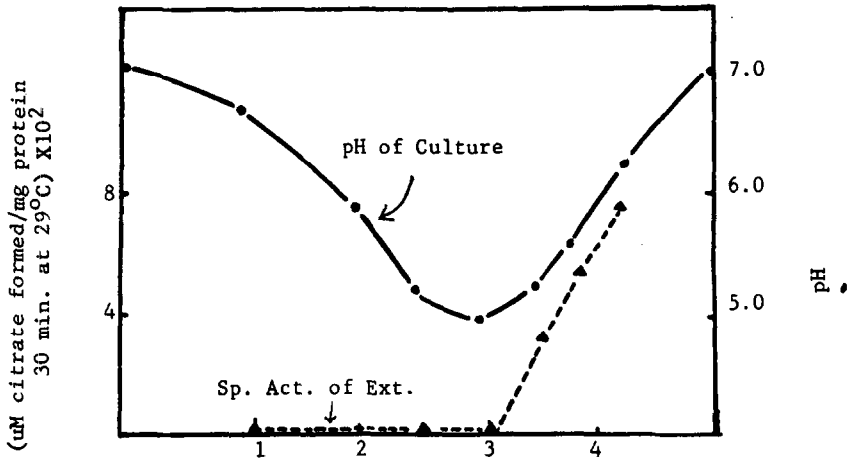

Figure 1. Citrate Formation in Extracts as a Function of Age of the Culture.

Reaction Mixture 1.0ml.: 0.1M tris buffer pH 7.6, 0.3M K-acetate, 0.02M OAA, 0.01M ATP, 0.001M CoA, 0.01M $MgCl_2$, protein ≃ 5.0 mg. Incubated at 29°C.

It is suggested that utilization of acetate by vegetative cells of B. cereus strain T is blocked because of the inability of the cells to convert acetate to citrate. The system is present and functional in extracts of the cells harvested during early sporulation[2].

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[1] G. medium (per cent W/V): $FeSO_4 \cdot 7H_2O$, 5 X 10^{-5}, $CuSO_4 \cdot 5H_2O$, 5 X 10^{-4}, $MnSO_4 \cdot H_2O$, 5 X 10^{-3}, $MgSO_4$, 2 X 10^{-2}, $CaCl_2 \cdot 2H_2O$, 8 X 10^{-3}, K_2HPO_4, 5 X 10^{-2}, $(NH_4)_2 SO_4$, 0.2 Yeast extract, 0.2 and glucose, 0.1.

[2] The cessation of multiplication of the organism has been taken to be the end of the vegetative phase and the beginning of the sporulation cycle (Gollakota & Halvorson 1960).

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