EVIDENCE FOR INDUCTION OF THE 2,3-BUTANEDIOL-FORMING ENZYMES IN *AEROBACTER AEROGENES*

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1. Introduction

Reynolds and Werkman [1] noticed that acetoin and butanediol formation was accompanied by a destruction of formed acetic acid when *Aerobacter indologenes* was grown on glucose. Later, Michelson and Werkman [2] suggested that acetate had to be present in an available form for the formation of these compounds.

Happold and Spencer [3] observed that acetate stimulated the formation of acetoin in cell-free extracts of *A. aerogenes*, from pyruvate, and it was shown by Störmer [4,5] that acetate acts as an activator for the pH 6 acetolactate-forming enzyme. A preliminary report of some of the results presented in this paper has been reported [4].

2. Experimental

The organism used throughout this work was wild type *A. aerogenes* strain 1033 [6]. In addition, two mutants of *A. aerogenes* A-15 were employed, namely the strains IV-2, and 45-III: both valine-isoleucine requiring mutants, which also were deficient in the pH 6 acetolactate-forming enzyme activity (E_1), according to Halpern and Even-Shoshan [7]. They were also found to have reduced butyleneglycol dehydrogenase activity (E_3), and strain 45-III, in addition, had a block in acetolactate decarboxylase activity (E_2).

The bacteria were grown in minimal media [8], supplemented with trace elements [9], and distilled water was used. To the cultures of the auxotroph mutants, L-leucine, 12.5 μg per ml, DL-valine, 25 μg per ml, and DL-isoleucine, 25 μg per ml, were also added [7]. 1% Bactodextrose was used as the source of carbon. Extracts were prepared by ultrasonic oscillation, and E_1 and E_2 were assayed as described [6], and E_3 was measured by following the reduction of NAD in the presence of 2,3-butanediol at room temperature [10]. Protein was determined colorimetrically [11].

Cocarboxylase, sodium pyruvate, and NAD were purchased from the Sigma Chemical Company, 5-fluorouracil from Calbiochem, and DL-*p*-fluoro-phenylalanine from Mann Research Laboratories.

3. Results and discussion

Table 1 shows the effect of various organic acids on the appearance of the butanediol-forming enzymes in *A. aerogenes* strain 1033, and that acetate markedly stimulated the formation of the enzymes. The following compounds were inactive: acetoin, 2,3-butanediol, lactate, acetamide, glyoxylate, ethanol, succinate, and acetyl-phosphate. The stimulatory effect of acetate is quite specific, as can be seen from table 1. Pyruvate caused some stimulation, but this effect might be of secondary nature, since pyruvate is easily converted to acetate, and no stimulation was observed with lactate. The other homologues of acetate examined were slightly stimulatory. Acetamide did not stimulate, indicating that the carboxyl group is essential for this effect. If glucose was replaced by citrate or glycerol as the carbon source, no enhancement of enzyme activities could be detected in the presence of acetate, indicating that factors in addition to acetate are necessary for this effect. When the cultures, in addition to

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Table 1
Effect of different organic acids on the formation of the butanediol-forming enzymes.

Addition to media	Enzyme activities (%)		
	E_1	E_2	E_3
Acetate	100	100	100
Formate	14	23	32
Propionate	21	22	23
Butyrate	14	16	16
Isobutyrate	23	21	21
Pyruvate	51	44	48
Monochloroacetate	16	26	23
None	8	11	11

The cultures were incubated at 37° with gyrotory shaking, and the cells were harvested in their exponentially growing phase when pH had dropped from 7.0 to 6.6, and resuspended in fresh medium pH 7.0. 40 ml portions were divided on flasks containing 4 mmoles of the test compound, previously adjusted to pH 7.0 with 1 M NaOH. The flasks were shaken at 37° for 60 minutes, rapidly cooled, and the cells were harvested and the specific activities of the enzymes involved in the formation of butanediol determined. E_1 is the pH 6 acetolactate-forming enzyme, E_2 is acetolactate decarboxylase, and E_3 is the butyleneglycol dehydrogenase.

Table 2
Effect of acetate on the induction of the butanediol-forming enzymes.

	Specific activities					
	E_1		E_2		E_3	
Acetate (100 mM)	+	−	+	−	+	−
Strain						
1033	167	4.0	113	1.4	240	0.7
IV-2	1.4	2.0	124	2.1	8.6	0.6
45-III	1.8	2.7	1.0	1.4	1.0	0.4

For conditions, see table 1. The specific activities are expressed as follows: E_1 and E_2 in μmoles product formed per mg protein per hour, and E_3 in μmoles $NADH_2$ formed per mg protein per minute.

acetate, contained 100 μg per ml of either DL-*p*-fluoro phenylalanine or 5-fluorouracil, the appearance of the enzymes was altered. The presence of the former compound greatly reduced the activities of all enzymes, and fluorouracil did not strongly inhibit the formation of E_1 and E_2, whereas little activity of E_3 could be detected.

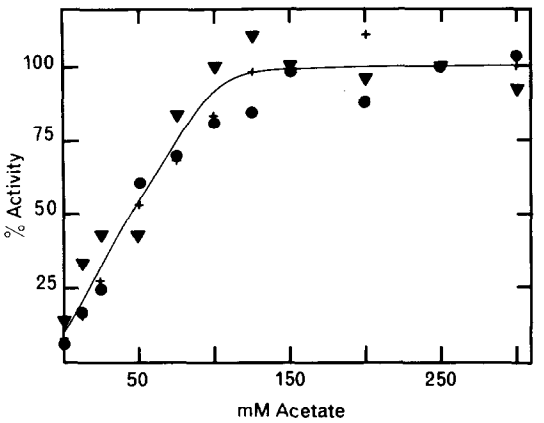

Fig. 1. Effect of acetate concentration on the formation of the butanediol-forming enzymes, E_1 (•), E_2 (+), and E_3 (▾). For experimental conditions, see table 1.

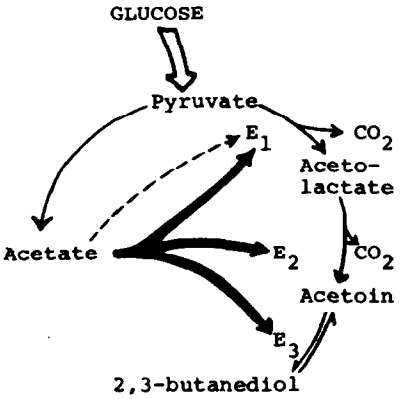

Fig. 2. The effect of acetate upon the formation of acetolactate, acetoin, and 2,3-butanediol. Black heavy lines show the induction of the respective enzymes by acetate, and the dashed line the activation of E_1 by acetate.

These results indicate that acetate induces the formation of these enzymes in *A. aerogenes*. That the induction is of coordinated nature is suggested by the data in table 2. The amount of E_2 increases in the same proportion in IV-2, which lacks E_1, as in strain 1033 in the presence of acetate. Some stimulation was also observed for E_3. It is possible that another metabolite appears in the cell, in response to the presence of acetate, and that this compound induces the butanediol-forming enzymes.

In fig. 1 the enzyme activities are plotted as a function of acetate concentration, and the observed values are scattered around the curved line in the figure. All the three enzymes reach a plateau at about 150 mM acetate, and the appearance of the enzymes does not change for acetate concentrations up to 300 mM. The effect of acetate upon the activation and induction of the butanediol-forming enzymes is summarized in fig. 2.

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