The Effects of Glucose, Acetate, and Malonate Upon Fatty Acid Distribution in *Aspergillus niger.*

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Abstract

Submerged cultures of *Aspergillus niger* were grown in media containing different concentrations of glucose, sodium acetate, or sodium malonate. Varying concentrations of glucose with or without added acetate did not alter the distribution of fatty acids produced by 120 hour cultures.

Increasing the concentrations of sodium acetate in the 5 per cent glucose medium caused the percentage of saturated fatty acids to increase from 20 per cent to 68 per cent. However, aerated stir cultures containing 2.5 per cent acetate produced the normal amount of unsaturated fatty acids with linolenic acid representing the largest component. In the control cultures linoleic is the major unsaturated fatty acid.

The high acetate cultures also differed from the control in that their growth was markedly inhibited, and the sharp decrease in pH which is characteristic of *Aspergillus* cultures was not observed.

Similar but not as pronounced effects were observed when sodium malonate was added to the 5 per cent glucose medium.

Introduction

Research in recent years has indicated that there are at least two pathways for the biosynthesis of fatty acids. The acetyl-coenzyme A pathway has been observed in intact mitochondria isolated from plants (1, 2, 3, 8, 13, 19, 21) and mammals (6, 7, 18). The malonyl-coenzyme A pathway, which occurs outside of the mitochondria, has been identified in many plant and animal tissues (20, 21). In soybeans (9), isolated plant leaves (10), and yeast (5) the long chain fatty saturated fatty acids are subsequently converted to unsaturated fatty acids by an oxygen dependent enzyme system.

Members of the Class Ascomycetes, with fatty acid compositions similar to those found in higher plants (15), have been used to study the biosynthesis of fatty acids in plants and plant-like organisms (4, 11, 17). Matto (12) reported that fatty acid synthesis in *Aspergillus niger* was reduced but not completely inhibited when avidin, a biotin inhibitor, was added to the medium, suggesting that fatty acids are synthesized by a pathway other than that using malonyl-coenzyme A. If the alternate pathway utilizes acetate, it should be possible to stimulate fatty acid synthesis by this pathway by increasing the amounts of acetate in the presence of a carbohydrate source in the medium. However, Romano and Kornberg (14) have reported that cultures of *Aspergillus nidulans* grown in a medium containing 100 mM of sodium acetate and 100 mM of glucose were markedly inhibited in growth and their ability to incorporate hexoses. The fatty acid distribution of the inhibited cultures was not reported.

In this study the effects of adding increasing amounts of sodium acetate and sodium malonate to the culture medium of *Aspergillus niger* were investigated.

Experimental Procedure

Aspergillus niger (LBa 376) was maintained on potato dextrose agar slants. Spores were lightly scraped and washed from the slants with sterile medium. One ml of the spore suspension was used to inoculate 100 ml of sterile culture medium. One set of experiments used a medium containing from 0 to 5 g glucose and 0.2 g KH_2PO_4; 0.2 g $MgSO_4.7H_2O$; 0.2 g NH_4NO_3; 5 g $NaC_2H_3O_2$; and 100 ml H_2O. In another, the medium contained 5 g glucose, 0.2 g NH_4NO_3; 0.2 g KH_2PO_4; 0.05 g $MgSO_4$; 100 ml H_2O and from 0 to 15 g of acetate or malonate. Control cultures contained no acetate or malonate. Cultures were incubated for 120 hours on a reciprocating shaker (180 revolutions per minute). Stir cultures were aerated and stirred for 120 hours. Cultures used for time studies were stirred and aerated in 600 ml of medium in a 1l flask.

At the end of the incubation period or at selected time points the mycelium was harvested by filtration through glass wool, resuspended in 15 ml absolute methanol, and sonified for 3 min (J-17A Sonifier, Branson Sonic Co.). The sonicate was diluted with an equal volume of 15% methanoic KOH, refluxed for 2 hours, and acidifed with concentrated HCl. The fatty acids were extracted with hexane, washed with distilled water, and filtered through anhydrous sodium sulfate. After removing the solvent on a rotary evaporator, the fatty acids were methylated with diazomethane (14). The methyl esters were isolated on silica gel G thin layer chromotography plates, and separated, identified, and quantified by gas liquid chromatography using a 10-foot glass column packed with 5% DEGA on 60/80 mesh chrom GA/W; column temperature, 190°C; gas flow, 70 ml/min (Varian Aerograph 90-P).

Results and Discussion

Although Woodbine *et al.* (21) reported that the addition of increasing amounts of carbohydrates to the medium stimulated total fatty acid synthesis in *Aspergillus niger*, our results indicated that increasing the amount of glucose in the presence of 5% acetate did not affect fatty acid distribution in 120-hour cultures (Fig. 1).

The normal decrease in pH observed in cultures grown without acetate did not occur in cultures grown in a medium containing 5% glucose and 2.5% or 5% sodium acetate. Growth of the mycelium was markedly inhibited at these concentrations and completely inhibited at 15%. Romano and Kornberg (14) reported that the uptake of glucose in cultures grown in a medium with 100 mM acetate decreases as much as 80%. They suggested that acetyl-coenzyme A was responsible for the inhibition of hexose uptake. Apparently the acetate also inhibits the secretion of citric and gluconic acids which is characteristic of normal cultures.

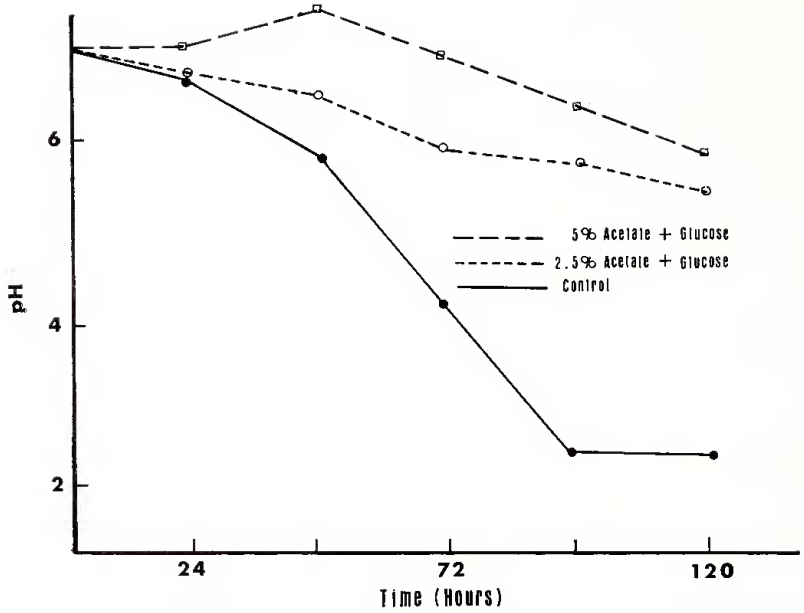

FIGURE 1. *Changes in pH during growth of* Aspergillus niger *on different substrates.*

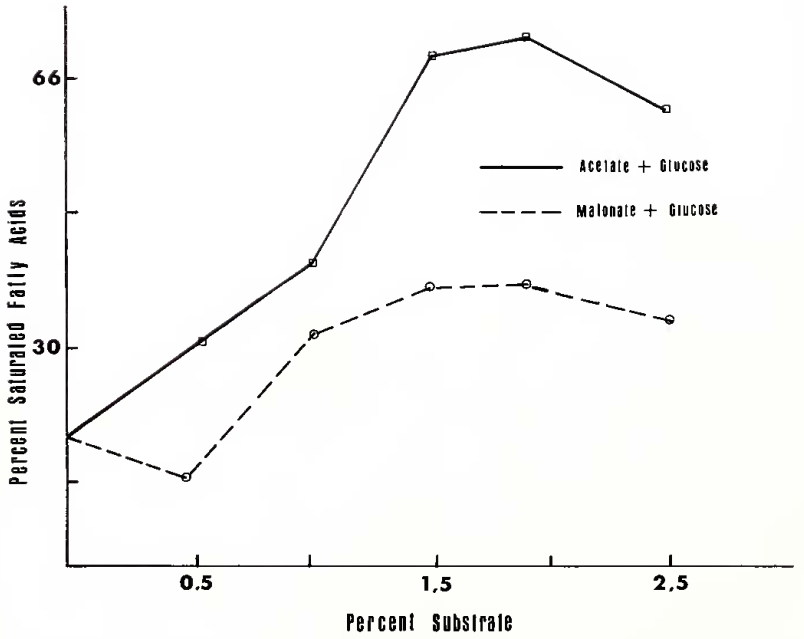

FIGURE 2. *Per cent saturated fatty acids recovered from* Aspergillus niger *grown in the presence of acetate and malonate.*

TABLE 1. *The effects of growing cultures two different ways*[1].

Culture	Saturated	Unsaturated
Control, Shake	20	80
2.5% Acetate, Shake	64	36
2.5% Acetate, Stir and Aerate	25	75

[1]Harvested after 120 hours growth at 28°C.

The per cent saturated fatty acids produced by shake cultures grown in a medium containing 2.5% sodium acetate was 48% higher than that produced by control cultures (Fig. 2). A similar, but smaller (16%) increase was effected by the addition of sodium malonate. This effect could be reversed by stirring and aerating high acetate cultures (Table 1). Upon aeration the percentage of unsaturated fatty acids in the acetate culture was similar to the control.

However, when acetate was included in the medium, linolenic acid represented the largest component while linoleic acid was highest in the control (Table 2). Whether this difference in the distribution of unsaturated fatty acids was due to the increased availability of the acetate or the decreased availability of the glucose is not clear.

TABLE 2. *Per cent distribution of the major Fatty acids recovered from* Aspergillus niger *in a submerged medium containing varying concentrations of acetate*[1].

Per cent Acetate	Per cent Glucose	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3
0.0	5	2	1	10	t	1	24	54	t
2.5	5	2	t	18	4	5	11	28	28
5.0	5	10	1	11	2	1	4	28	40
7.5	5	16	t	19	9	2	6	23	35
10.0	5	5	1	13	7	2	6	28	34
12.5	5	15	5	9	25	4	6	15	20
15-35	5	No Growth							

[1]Grown at 28°C for five days in aerated stir cultures.

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