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Effects of Temperature Variation on the Fatty Acid Composition of *Candida utilis*

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The fatty acid compositions of various cultures of the yeast *Candida utilis* NCYC 321 were analyzed by gas-liquid chromatography of the methyl esters obtained from the lipids in chloroform-methanol extracts of the cells. Over a wide range of growth conditions *C. utilis* contained mainly 16:0, 16:1, 18:1, 18:2, and 18:3 fatty acids in variable proportions. The most variable aspect of the fatty-acid composition of *C. utilis* was in the relative proportions of 18:1, 18:2, and 18:3 acids. During batch growth at 30 C, the relative proportions of 18:3 decreased, whereas 18:1 increased as the cultures aged. Batch cultures grown at low temperatures maintained higher proportions of 18:3 acids than cultures grown at 30 C. When stationary cultures were replenished with fresh medium under aerobic conditions, there was an abrupt increase in the proportion of 18:3 with a concomitant decrease in 18:1 acids in the cells. The fatty acid composition of cells grown in a chemostat at 30 C did not vary much in response to changes in either the growth rate or the growth-limiting substrate. Chemostat-grown cells contained highest proportions of 18:3 acid when grown under conditions of glucose-limitation at low temperatures.

It has been widely reported that microorganisms synthesize increased proportions of unsaturated fatty acids when growth takes place at temperatures below the optimum (5, 6, 8, 9). In addition it has been shown that the dissolved oxygen content (1, 3, 7) and growth rate (4, 11) of cultures influences the fatty acid composition of microorganisms. Several studies have been made on the effects of the aforementioned variables on the fatty acid composition of *Candida utilis* grown in continuous cultures (1, 3, 4). In this study, the influences of temperature on the fatty acid composition of batch-grown and chemostat-grown cultures of *C. utilis* were compared. Temperature variations were used to control directly the rate of batch growth, whereas chemostat cultures were grown at a fixed rate at different incubation temperatures. It was of interest to determine whether the effect of temperature on the fatty acid composition of *C. utilis* could be modified by manipulating culture conditions.

MATERIALS AND METHODS

Organism. The strain of *C. utilis* NCYC 321 used in this study was maintained as described previously (13).

Media. For batch growth, liquid media contained glucose, 10.0 g; $(NH_4)_2SO_4$, 5.0 g; KH_2PO_4, 7.0 g; $MgSO_4 \cdot 7H_2O$, 1.0 g; $CaCl_2 \cdot 6H_2O$, 0.25 g; nicotinic acid, 2.0 mg.; pyridoxine-hydrochloride, 1.0 mg; meso-inositol, 10.0 mg; thiamine-hydrochloride, 1.0 mg; and D-biotin, 0.01 mg; per liter of water. The medium was adjusted to *p*H 5.5 and dispensed in 1-liter portions contained in round 2-liter flat-bottom flasks. For continuous culture, the media used were as described by Brown and Rose (2).

Culture conditions. Aerobic batch cultures were magnetically stirred at a fixed speed and were held in a water bath at the temperatures specified. For anaerobic growth, oxygen-free nitrogen was flushed through the culture flasks at a rate of 60 ml/hr. Continuous cultures were grown in a 0.5-liter chemostat as originally described by McMurrough and Rose (12) and later used for temperature studies by Brown and Rose (2).

Measurement of growth. The cell concentration (milligrams, dry weight, per milliliter) of the cultures was measured as previously described (12). The specific growth rate, k, of batch cultures was computed from the formula

$$k = 2.303 (\log x_2 - \log x_1)/(t_2 - t_1)\ hr^{-1}$$

where x_1 and x_2 are cell concentrations at times (hours) t_1 and t_2, respectively.

Analyses of cells. Cells were harvested by centrifuging cultures at 2,000 × *g*. Lipids were then extracted from twice-washed cells and analyzed for fatty acids as previously described (3). Methyl esters of fatty acids were prepared by direct methanolysis of chloroform-methanol extracts from the yeast cells. The esters were separated by gas-liquid chromatography in a column containing polyethylene-glycol succinate on chromosorb-W. Methyl esters were identified by com-

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paring their retention times with those of known mixtures. Fatty acids are designated $x:y$ where x is the number of carbon atoms and y the number of double bonds per molecule.

Glucose measurements. The glucose contents of the supernatant fluids remaining after centrifuging cultures at 2,000 × g for 10 min were determined with Glucostat reagent (Worthington Biochemical Corp., Freehold, N.J.).

RESULTS

Fatty acid composition of Candida utilis. Assay of the constituent fatty acids of *C. utilis* revealed that the yeast contained predominantly esters of palmitic (16:0), palmitoleic (16:1), oleic (18:1), linoleic (18:2), and linolenic (18:3) acids in variable proportions. Esters of stearic acid (18:0) and other fatty acids never accounted for more than 2% of the total fatty acid content and are not stated in our results.

Effect of temperature on the fatty acid composition and growth of Candida utilis in batch culture. The effect of temperature on the specific growth rate, k, was examined by inoculating batches of medium with cells from slope cultures of *C. utilis* to a concentration of 0.02 mg (dry weight) of cells per ml of culture and incubating such cultures aerobically over the temperature range 5 to 30 C at 5 C intervals. The growth of each culture was followed by periodic sampling, and large samples (50 mg) were collected from each culture for fatty acid analysis when the concentration of cells had reached 0.2 and 1.0 mg (dry weight) per ml of culture. At these concentrations, the cultures corresponded to early and late-exponential periods of growth, respectively. The effect of temperature on specific growth rate and fatty acid composition is shown in Tables 1 and 2. Temperature differences had a profound influence on specific growth rate but did not significantly alter the fatty acid composition of cells from early exponential-phase cultures. In contrast, the fatty acid compositions of cells from late exponential cultures were markedly different. A decrease in growth temperature down to 10 C invariably resulted in increased proportions of palmitate, palmitoleate, and linolenate at the expense of oleate and linoleate. The glucose concentration of cultures harvested in their late period of exponential growth was approximately 30% of the initial concentration (Table 2).

TABLE 1. *Effect of incubation temperature on the specific growth rate of cultures and on the fatty acid composition of early exponential-growth cultures of Candida utilis*

Incubation temp (C)	Specific growth rate[a]	Fatty acid composition[b]				
		16:0	16:1	18:1	18:2	18:3
30	0.510	12.0	6.0	14.0	37.3	27.1
25	0.400	11.9	4.5	13.5	40.7	26.4
20	0.252	11.7	4.6	13.1	41.1	26.5
15	0.131	11.1	3.0	13.2	43.2	26.3
10	0.060	11.9	3.1	12.9	43.7	24.5
5	0.006	10.9	2.3	15.0	45.2	22.6

[a] Expressed as values per hour.

[b] Values quoted are expressed as percentages of the total fatty acids.

TABLE 2. *Glucose concentration and cellular fatty acid composition of late exponential-growth cultures of Candida utilis grown at different temperatures*

Incubation temp (C)	Glucose concn (mg/ml)	Fatty acid composition[a]				
		16:0	16:1	18:1	18:2	18:3
30	3.3	18.2	4.9	39.2	34.0	2.3
25	2.7	19.5	6.3	35.1	27.9	6.0
20	3.4	20.1	7.9	32.1	27.3	7.3
15	2.9	23.9	11.9	28.8	21.1	9.4
10	2.9	27.3	16.7	21.2	17.4	12.3
5	3.9	18.6	13.3	17.4	17.0	28.7

[a] Values quoted are expressed as percentages of the total fatty acids.

Effect of temperature and growth rate on the fatty acid composition of Candida utilis in chemostat culture. *C. utilis* was grown under steady-state conditions in a chemostat over a range of dilution rates at 30 C with either the glucose source or the nitrogen source (NH_4^+) as the growth-limiting substrate. Variations in the growth rate of the cultures in the steady state (equal to dilution rate) from 0.35 to 0.05 hr^{-1} produced only minor alterations in the fatty acid composition of *C. utilis* (Table 3). In addition, the nature of the growth-limiting substrate had little influence on fatty acid composition.

Cultures of *C. utilis* were also grown at a fixed rate (0.1 hr^{-1}) at different temperatures under conditions in which either glucose or the nitrogen source limited growth (Table 4). It was found that the nature of the growth-limiting substrate distinctly influenced the effect of temperature on the fatty acid composition of *C. utilis*. Under conditions of glucose limitation, a decrease in incubation temperature caused decreases in the proportions of palmitate, oleate, and linoleate, whereas the proportions of palmitoleate and linolenate increased. Under conditions of nitrogen source limitation, the proportions of palmitoleate and linoleate varied in a similar manner; however, the proportions of palmitate and oleate increased and linolenate remained almost stationary, as the incubation temperature was lowered.

The effects of temperature on continuous cultures do not find a precise analogy with the results obtained with batch culture (Tables 1, 2).

TABLE 3. *Effect of growth rate on the fatty acid composition of continuous cultures of Candida utilis grown at 30 C*

Specific growth rate[a]	Growth-limiting substrate	Fatty acid composition[b]				
		16:0	16:1	18:1	18:2	18:3
0.35	Glucose	17.2	3.5	20.5	40.3	16.5
0.28		16.5	2.5	22.1	44.5	12.4
0.20		19.5	2.5	24.4	37.1	14.0
0.10		16.1	6.3	25.5	47.1	7.9
0.35	Nitrogen	17.2	5.3	17.4	38.3	19.7
0.20	source	17.3	3.0	24.5	37.1	16.1
0.10		17.9	2.3	25.7	39.3	13.1
0.05		18.3	1.0	26.5	43.6	9.6

[a] Expressed as values per hour.

[b] Values quoted are expressed as percentages of total fatty acids.

The fatty acid composition is but one measurable expression of the physiological state of a culture. Accordingly, variations in the gross fatty acid composition might arise due to some alteration in the cell contents of mitochondria, storage vesicles, etc., in response to environmental changes. In a chemostat, the physiological steady states which are experimentally obtainable are restricted to a relatively narrow range in which the cultures grow under conditions of nutrient limitation. A batch culture, in contrast, is a heterogeneous cell population which can progress through a sequence of physiological states, defined in part by ancestry and partly by the immediate environment. As a consequence it is not possible in a single-stage chemostat to duplicate and maintain the full spectrum of physiological states that might arise during batch cultivation.

Changes in fatty acid composition during the growth of batch cultures at different temperatures. Marked differences between the fatty acid compositions of cells harvested from cultures in their early and late periods of exponential growth were evident. It was of interest, therefore, to examine in more detail the extent of the variations in fatty acid composition during batch growth at different temperatures.

Batch cultures were inoculated as described previously and were incubated aerobically in water baths at either 30, 15, 10, or 5 C. Periodically, samples (5.0 ml) of culture were taken for the measurement of cell concentration, and larger samples (50 mg of cells) were harvested at appropriate intervals for fatty acid analysis. The fatty acid composition of *C. utilis* varied considerably during the culture cycle at all temperatures (Fig. 1). In general, the early and middle periods of exponential growth were characterized by an increase in the proportions of palmitate, palmitoleate, and oleate, whereas the proportions of linoleate and linolenate decreased. The fatty acid composition of cells from cultures grown to their late-exponential-growth phase (1.0 mg, dry weight, of cells per ml of culture) at different temperatures were quantitatively dissimilar, although similar trends in composition were recognizable as the cultures aged. A decreased incubation temperature, in general, resulted in larger proportions of palmitate, palmitoleate, and linolenate at the expense of oleate and linoleate.

Growth and fatty acid composition of "glucose-replenished" cultures of C. utilis. Major variations in the fatty acid composition of *C. utilis* were observed during the period of active growth in batch culture regardless of the temperature of incubation. These variations underscore changes in the metabolism of the cells during growth of the cultures. In the late period of exponential growth, the glucose content of the cultures had decreased to about one third of the initial concentration (Table 2). It was of interest, therefore, to determine whether the changes in fatty acid composition during cell growth might arise as consequences of the depletion of available glucose. It was reasoned that if this were so, the replenishment of cultures with fresh medium might promote a reversal of the observed changes in fatty acid composition.

A "glucose-starved" culture of *C. utilis* was prepared by first inoculating medium with cells from a slope culture as described previously. This culture was incubated aerobically for 36 hr at 30 C. At this time, the cell concentration was 2.5 mg (dry weight)/ml of culture, and the glucose concentration was estimated at about 50 μg/ml of culture. About 50 mg of cells were harvested for fatty acid analysis, whereas 40-ml samples of culture were diluted by addition to 1-liter batches of fresh medium, held at 30 C in a water bath. These glucose-replenished cultures were incubated either aerobically or anaerobi-

TABLE 4. *Effect of incubation temperature on the fatty acid composition of continuous cultures of Candida utilis grown at a specific growth rate of 0.1 hr^{-1}*

Incubation temp (C)	Growth-limiting substrate	Fatty acid composition[a]				
		16:0	16:1	18:1	18:2	18:3
30	Glucose	16.1	6.3	25.5	47.1	7.9
20		14.9	13.3	17.4	25.1	27.3
15		8.3	13.7	18.9	22.0	38.0
30	Nitrogen	17.9	2.3	25.7	39.3	13.1
20	source	18.2	8.0	27.3	29.7	14.6
15		20.3	9.9	31.3	25.6	11.0

[a] Values quoted are expressed as percentages of the total fatty acids.

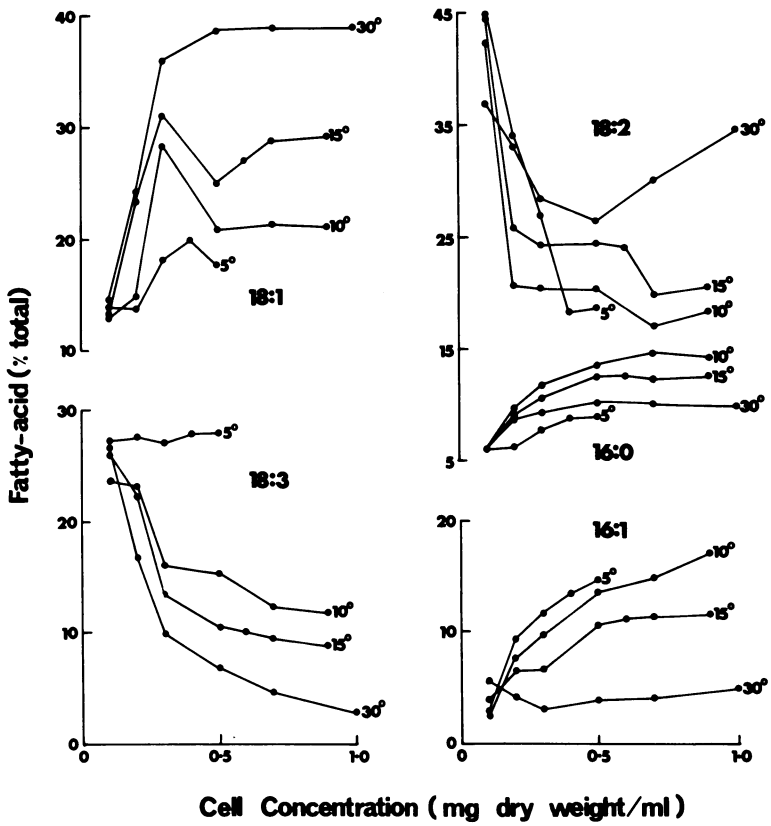

FIG. 1. *Changes in the fatty acid composition of Candida utilis during aerobic batch growth at 5, 10, 15, and 30 C. Individual fatty acids are denoted as 16:0 (palmitic), 16:1 (palmitoleic), 18:1 (oleic), 18:2 (linoleic), and 18:3 (linolenic) and are expressed as a percentage of the total fatty-acids.*

cally, and samples of culture were taken periodically for growth measurement and fatty acid analysis. When glucose-starved cells were replenished with fresh medium and aerobically incubated, a 6-hr lag period preceded the commencement of exponential growth. Exponential growth continued until the cell concentration was about 0.9 mg (dry weight) per ml of culture. During the period of active growth, the fatty acid composition of the cells varied markedly. Pronounced changes in the proportions of oleate and linolenate occurred, whereas the proportion of palmitoleate remained at a low, almost constant, level (Fig. 2). Under anaerobic conditions, glucose-replenished cultures commenced exponential growth at a rate of 0.21 hr^{-1}. During the period of growth, some variations in the cellular fatty acid compositions were evident. The pattern of change observed differed in certain respects from that of aerobic cultures grown at 30 C. Under anaerobic growth conditions, *C. utilis* contained a much smaller proportion of linolenate and a much higher proportion of palmitoleate as compared with aerobically grown cells (Fig. 2). Moreover, the proportion of oleate in anaerobically grown cells decreased gradually throughout the growth of the culture in contrast with the sharp fluctuation observed early in the exponential growth phase of aerobic cultures. Throughout the growth period of both cultures, there was little difference in the relative proportions of palmitate and linoleate. Finally, the combined effects of glucose replenishment and a temperature step-down were investigated using a glucose-starved culture. Glucose-starved cultures were replenished with precooled medium as described above and incubated aerobically at 10 C. A 24-hr lag period was evident before growth was resumed. During this lag period, the fatty acid composition of the cultures changed considerably; however, after growth had commenced, changes in fatty acid composition were less evident. Changes in the proportions of oleate and linolenate (Fig. 3) during the growth of glucose-replenished cultures at 10 C were quantitatively the most significant.

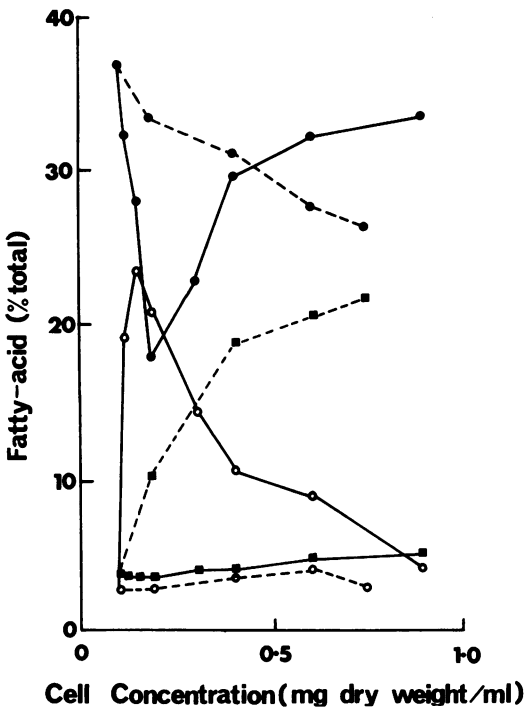

FIG. 2. *Changes in the cellular contents of palmitoleic* (■), *oleic* (●), *and linolenic* (○) *acid esters during the aerobic* (——) *and anaerobic* (- - -) *growth of glucose-replenished batch-cultures of Candida utilis at 30 C.*

DISCUSSION

Regardless of the incubation temperature, the fatty acid composition of *C. utilis* was found to vary during the period of exponential growth in batch culture. In general, variations in fatty acid composition were minimized when incubation temperatures and growth rates were low, so that higher proportions of polyunsaturated acids were maintained as the cultures aged. Replenishment of stationary-phase cultures, grown aerobically at 30 C, with fresh medium resulted in an abrupt increase in the proportion of linolenate with a corresponding decrease in the proportion of oleate (Fig. 2). This change in fatty acid composition was dependent on the availability of oxygen but was only slightly influenced by the incubation temperature. During growth of glucose-replenished cultures at 10 C, the fatty acid composition with respect to oleate and linolenate (Fig. 3) was relatively stable after the early period of exponential growth, in contrast to the variability observed in cultures grown at 30 C (Fig. 2).

The exponential-growth rates of batch cultures were varied by altering the incubation temperatures (Table 1), in contrast to the chemostat cultures in which growth rates were controlled by the rate of replacement of nutrient. As a consequence, the chemostat cultures were not grown at the maximum rates compatible with a given temperature. It has already been suggested that chemostat cultures might respond in some unique manner to variations in temperature (3); thus, for this and other reasons, it is no surprise to find that the fatty acid compositions of chemostat cultures are dissimilar to those of batch cultures. Over the relatively narrow range of growth rates studied the fatty acid compositions of steady-state cultures grown aerobically at 30 C did not vary much and were apparently not influenced significantly by the nature of the growth-limiting substrate. A slight decrease in the proportion of linolenate was observed when the growth rate was very low but the presence of a high glucose concentration was not essential for maintaining the proportion of linolenate above 10% (Table 3). When glucose was present in excess, only slight variations in the proportions of linolenate occurred as the incubation temperature of chemostat cultures was lowered (Table 4), whereas a large increase in linolenate was observed when glucose was growth limiting. The role of oxygen in these events is uncertain, since no attempt was made to control dissolved oxygen and the cul-

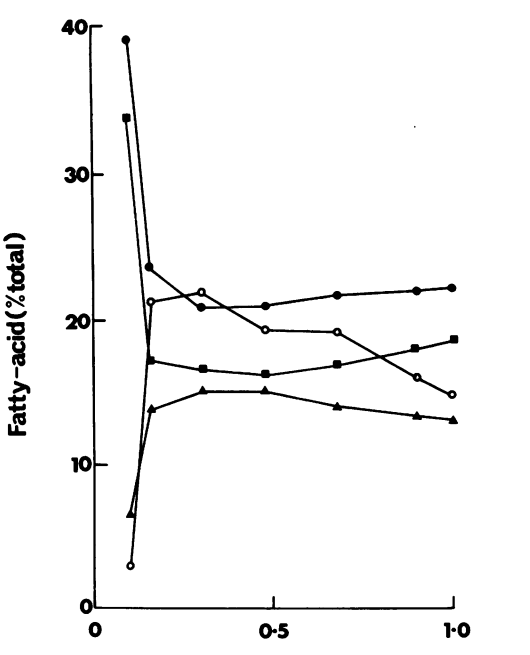

FIG. 3. *Changes in the cellular contents of palmitoleic* (▲), *oleic* (■), *linoleic* (●), *and linolenic* (○) *acid esters during the aerobic growth of glucose-replenished batch cultures of Candida utilis after a temperature step-down to 10 C.*

tures were assumed to be saturated under all conditions.

The fatty acid compositions reported for *C. utilis* grown in a turbidostat under conditions of high oxygen tension and high glucose concentration (1) are strikingly similar to those reported herein for early exponential-growth batch cultures. This similarity in fatty acid composition suggests that a more precise parallel perhaps exists between the pattern of behavior of batch and turbidostat cultures than has been presently observed with chemostat cultures of *C. utilis*. According to Babij, Moss, and Ralph (1), a preponderance of linolenate in *C. utilis* is associated with the formation of depot fat, for which high concentrations of glucose and oxygen in the culture are necessary. Although the data reported herein for batch cultures do not support this proposition in its entirety, they are in no way contradictory. The observed changes in fatty acid composition presently reported possibly arose through alterations in the cellular contents of storage and structural lipids. The effect of growth at suboptimal temperatures could be due to a retarded dissimilation of lipid reserves. As an alternative, it has been suggested (5, 6) that growth at low temperatures induces changes in fatty acid composition which might be indispensable for membrane structure and function. From this study, however, it seems that fluctuations in the fatty acid composition of *C. utilis*, particularly with respect to the proportions of linolenate and oleate, can arise in response to several environmental parameters. Our data do not permit a firm conclusion, but it seems possible that the maintenance of high proportions of polyunsaturated fatty acids is not a prerequisite for the growth of *C. utilis* at suboptimal temperatures. Experiments with aerobic batch cultures indicated that, regardless of the incubation temperature, high proportions (above 20%) of linolenate were present during the lag or early period of exponential growth. In contrast, high proportions of linolenate were formed in chemostat cultures only when growing under conditions of glucose limitation at low temperatures. In deference to our limited knowledge of the underlying mechanisms involved, it is not possible to correlate these findings with a definitive role for temperature in determining the fatty acid composition of *C. utilis*.

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