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Fatty Acid Composition of *Escherichia coli* as a Possible Controlling Factor of the Minimal Growth Temperature

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ABSTRACT

SHAW, MAXWELL K. (University of California, Davis), AND JOHN L. INGRAHAM. Fatty acid composition of *Escherichia coli* as a possible controlling factor of the minimal growth temperature. J. Bacteriol. **90**:141-146. 1965.—If *Escherichia coli* ML30 is shifted from 37 to 10 C during exponential growth in glucose minimal medium, a 4.5-hr lag results. During this lag, the proportion of unsaturated fatty acids increases in the cellular lipids. However, the adjustment of the fatty acid composition does not appear to be prerequisite to growth at 10 C. If shifts are made to 10 C into minimal medium containing glucose after starvation for glucose at 37 C for 0.5 and 16 hr, the lag periods at 10 C are 4.5 and 6 hr, respectively. Withholding glucose during the lag periods does not affect the duration of the lag periods, but no change in fatty acid composition occurs if glucose is not present. Supplementing the medium with glucose after the lag period permits immediate growth at 10 C; however, the fatty acid composition is still typical of cells grown at 37 C. It is concluded that the fatty acid composition of cells does not determine the minimal temperature of growth.

It has been proposed (Heilbrunn, 1924; Bělehrádek, 1931; Gaughran, 1947; Kates and Baxter, 1962) that the composition of the lipids of microorganisms may set the limits of temperature for growth. It is known that the lipids of *Escherichia coli* ML30 grown at low temperatures have a higher proportion of unsaturated fatty acids than the lipids of *E. coli* ML30 grown at higher temperatures (Marr and Ingraham, 1962).

Ng, Ingraham, and Marr (1962) showed that when *E. coli* ML30 growing exponentially at 37 C is shifted to 10 C, a 4.5-hr lag period results. During the lag period, there is no net ribonucleic acid (RNA), deoxyribonucleic acid (DNA), or protein synthesis, and no increase in numbers or size of cells, but respiration proceeds at a rate characteristic of a culture growing exponentially at 10 C (Shaw and Ingraham, *unpublished data*).

In this investigation, we determined, by gas-liquid chromatography, what changes occur in fatty acid composition of the lipids of *E. coli* ML30 during this lag period, to determine whether the lag results from the inability of cultures of *E. coli* to grow at 10 C until after adjustments in the composition of their lipids have been made. The results show that the unsaturation of fatty acids is not prerequisite for growth at 10 C, and suggest that the fatty acid composition of a bacterium is not directly related to the lower limit of temperature at which it will grow.

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MATERIALS AND METHODS

Media. The basal medium was medium 56 of Monod, Cohen-Bazire, and Cohn (1951). Glucose (0.1 or 0.01%) was added as carbon source.

Organism. E. coli ML30, obtained from J. Monod, was used in all experiments.

Growth conditions. Experiments in which growth rate alone was measured were done in vessels containing 150 ml of medium. If fatty acids were determined, cultures were grown in 8-liter bottles containing 5 liters of medium. The culture vessels were immersed in water baths at the stated temperature ±0.05 C and were aerated, by means of a sintered-glass thimble, with air saturated with water vapor at the temperature of the culture.

Temperature shifts were performed by shifting the culture vessel to the second bath and sparging immediately with air saturated with water vapor at the new temperature.

Measurement of growth. Growth was followed by periodic measurement of optical density in a 1-cm cell at 420 mμ with a Beckman model DU spectrophotometer. The dry weight of cells per milliliter was computed from the optical density, with the use of a standard curve prepared from dilutions of a culture growing exponentially at 37 C in glucose

basal medium. The specific growth rate, k, in hr^{-1}, was computed from the formula

$$k = \frac{dx}{x\,dt} = \frac{2.303(\log_{10} x_2 - \log_{10} x_1)}{t_2 - t_1}$$

in which x_1 and x_2 are the optical densities at times t_1 and t_2, respectively.

Analysis of fatty acids. Cells were harvested by centrifugation and washed twice with water. Approximately 0.2 g (dry weight) of cells was used for each determination. The methyl esters of the fatty acids were prepared according to the method of Marr and Ingraham (1962), and determined by gas-liquid chromatography with the use of a thermal conductivity detector and a column (0.25 inches by 9 ft; 0.63 by 274.3 cm) of 25% diethylene glycol-succinate polyester on fire brick at 189 C (Kaneshiro and Marr, 1961).

The fraction of each component was computed as described by Marr and Ingraham (1962).

RESULTS

Step-down experiments. A culture of *E. coli* ML30 growing exponentially in glucose basal

TABLE 1. *Fatty acid composition of Escherichia coli ML30 after shifting from 37 to 10 C**

Fatty acid	Time after shifting (hr)				
	0	1.5	4.5	18	24
Myristic	5.1†	5.3	4.7	5.2	5.3
Palmitic	38.6	30.6	27.7	25.1	25.0
Hexadecenoic	27.9	29.4	30.3	30.5	31.0
Methylene hexadecenoic	3.0	3.1	2.0	3.0	2.5
Octadecenoic	19.5	26.1	29.3	31.0	31.8
β-Hydroxymyristic	5.9	5.6	5.9	6.9	6.3
Hexadecenoic/palmitic	0.72	0.96	1.09	1.22	1.24
Octadecenoic/palmitic	0.51	0.85	1.06	1.24	1.27

* Glucose was present throughout the experiment.

† Results are expressed as per cent by weight, or ratios of per cent by weight.

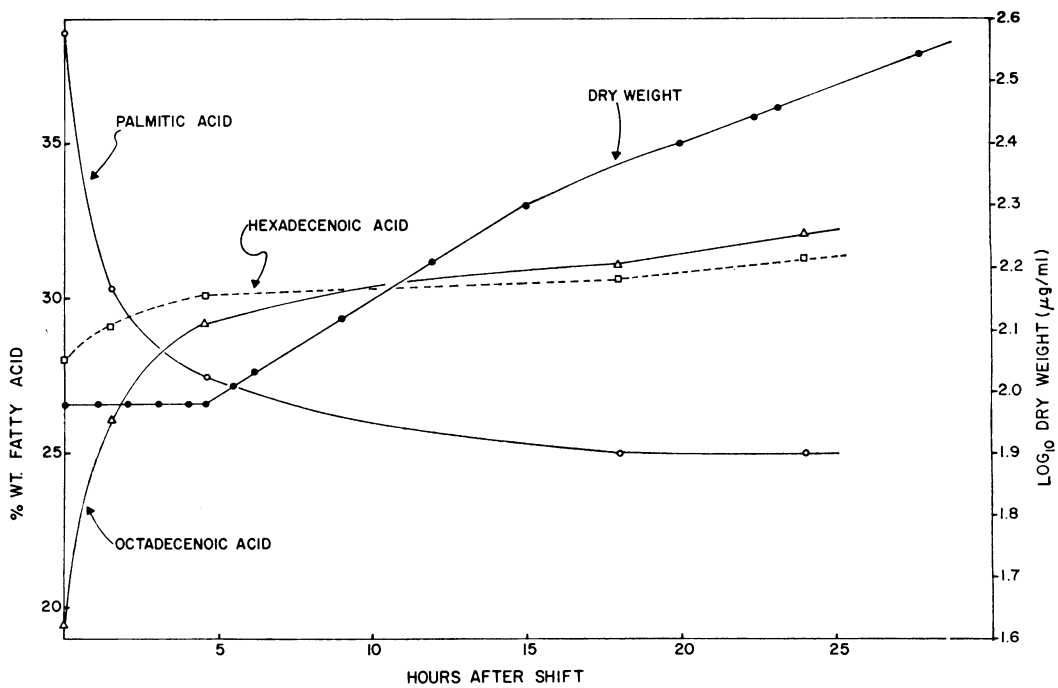

FIG. 1. *Change in dry weight and in proportions of palmitic, hexadecenoic, and octadecenoic acids after the shift to 10 C of a culture of Escherichia coli growing exponentially at 37 C.*

medium at 37 C (k = 0.90 hr^{-1}) was shifted to 10 C when the density of the culture reached 90 μg/ml (dry weight), and turbidity was followed. Samples (1 liter) were taken at 0, 1.5, 4.5, 10, and 24 hr after shifting, and the fatty acid composition was determined (Table 1). The composition of lipids at 0 hr was typical of cultures growing in the steady state at 37 C, and the composition of lipids at 24 hr was typical of cultures growing in the steady state at 10 C.

During this 24-hr period, the proportion of palmitic acid decreased (38.6 to 25.0%), hexadecenoic acid increased (27.9 to 31.0%), and octadecenoic acid increased (19.5 to 31.8%). The rate of these changes, as compared with the dry-weight increase, is shown in Fig. 1. During the lag of 4.5 hr, when most biosyntheses are stopped, a major change in fatty acid composition occurred. To establish whether the observed change in fatty acid composition is essential or coincidental to

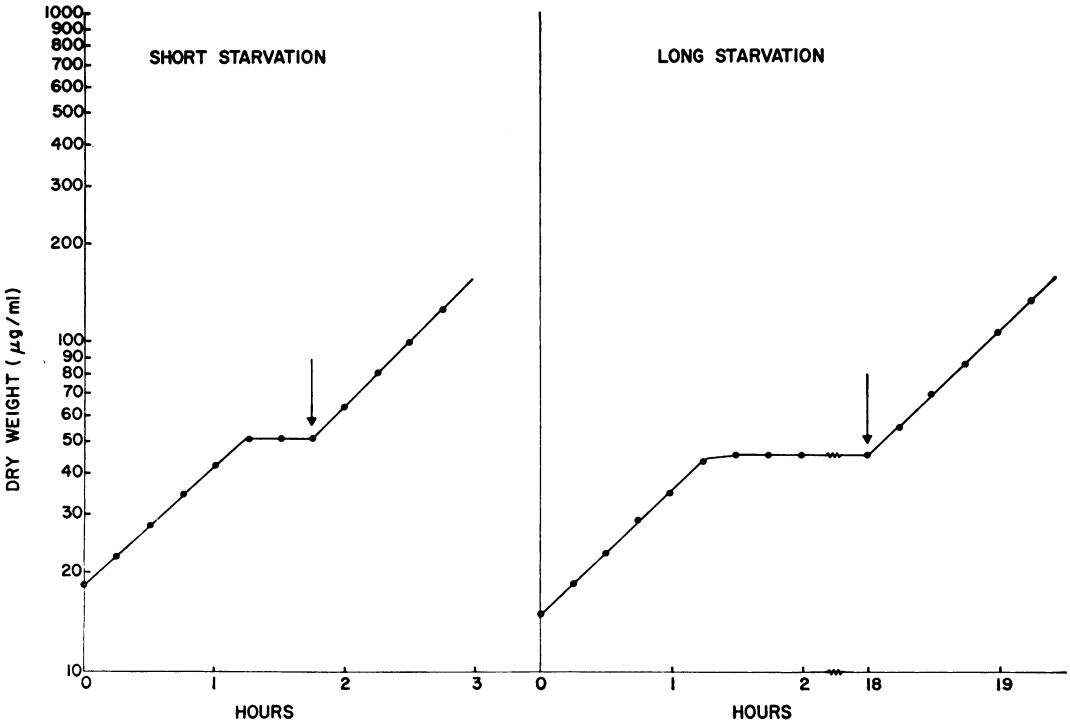

FIG. 2. *Growth of Escherichia coli at 37 C. Growth was limited at a dry weight of 50 μg/ml by exhaustion of glucose. Glucose (0.1%) was added to the culture at the times indicated by the vertical arrows.*

TABLE 2. *Fatty acid composition of Escherichia coli ML30 after shifting to 10 C, following 0.5 hr of carbon starvation at 37 C**

Fatty acid	Time after shifting (hr)				
	0	2	4.5	10	24
Myristic	6.3	5.4	6.7	3.0	3.9
Palmitic	38.8	37.9	37.2	26.8	22.9
Hexadecenoic	30.2	30.3	28.3	28.5	28.0
Methylene hexadecenoic	2.4	5.1	6.8	3.2	3.3
Octadecenoic	15.7	15.9	13.6	34.2	35.8
β-Hydroxymyristic	6.6	5.2	7.0	3.8	9.2
Hexadecenoic/palmitic	0.78	0.79	0.76	1.06	1.22
Octadecenoic/palmitic	0.40	0.42	0.37	1.28	1.56

* Glucose (0.1%) was added 4.5 hr after shift.

† Results are expressed as per cent by weight, or ratios of per cent by weight.

resumption of growth at 10 C, the culture was starved for carbon for various lengths of time at 37 C and was then shifted to 10 C.

Starvation experiments. Cultures were grown in 0.01% glucose medium, which supports a final yield of cells of about 50 μg/ml (dry weight). Cultures were starved after exhaustion of the carbon source for 0.5 and 16 hr before shifting to 10 C, and 0.1% glucose was added at the time of shifting. Control cultures at 37 C resumed exponential growth after starvation without a detectable lag (Fig. 2). After 16 hr of starvation, the lag at 10 C was increased only to 6 hr. Shifts to 10 C after starvation were then made into media without glucose, and 0.1% glucose was added after 4.5 hr in the case of short starvation and after 6 hr in the case of long starvation.

Fatty acid determinations were made under these conditions (Tables 2 and 3).

The rate of change of dry weight and of palmitic, hexadecenoic, and octadecenoic acids in the lipids is shown in Fig. 3 and 4.

TABLE 3. *Fatty acid composition of Escherichia coli ML30 after shifting to 10 C, following 16 hr of carbon starvation at 37 C**

Fatty acid	Time after shifting (hr)				
	0	2	6	10	24
Myristic	6.1	7.4	8.0	6.8	5.0
Palmitic	50.4	50.1	49.7	39.2	21.2
Hexadecenoic	12.6	12.5	13.8	17.5	29.1
Methylene hexadecenoic	7.9	7.9	8.7	6.1	3.3
Octadecenoic	8.0	10.4	9.0	23.1	36.3
β-Hydroxymyristic	12.0	9.5	10.7	6.9	8.9
Hexadecenoic/palmitic	0.25	0.25	0.28	0.45	1.37
Octadecenoic/palmitic	0.16	0.25	0.18	0.59	1.71

* Glucose (0.1%) was added 6 hr after shift.

† Results are expressed as per cent by weight, or ratios of per cent by weight.

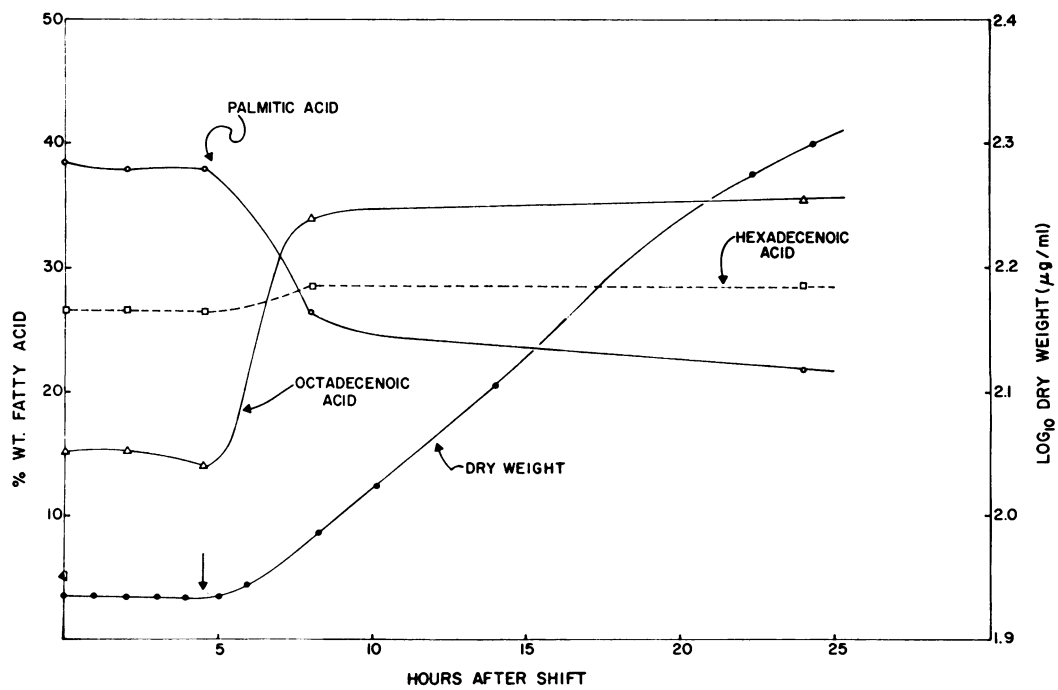

FIG. 3. *Change in dry weight and in proportions of palmitic, hexadecenoic, and octadecenoic acids after the shift of a culture of Escherichia coli to 10 C, following a period of 0.5 hr of starvation for glucose at 37 C. Glucose (0.1%) was added to the culture at the time indicated by the vertical arrow.*

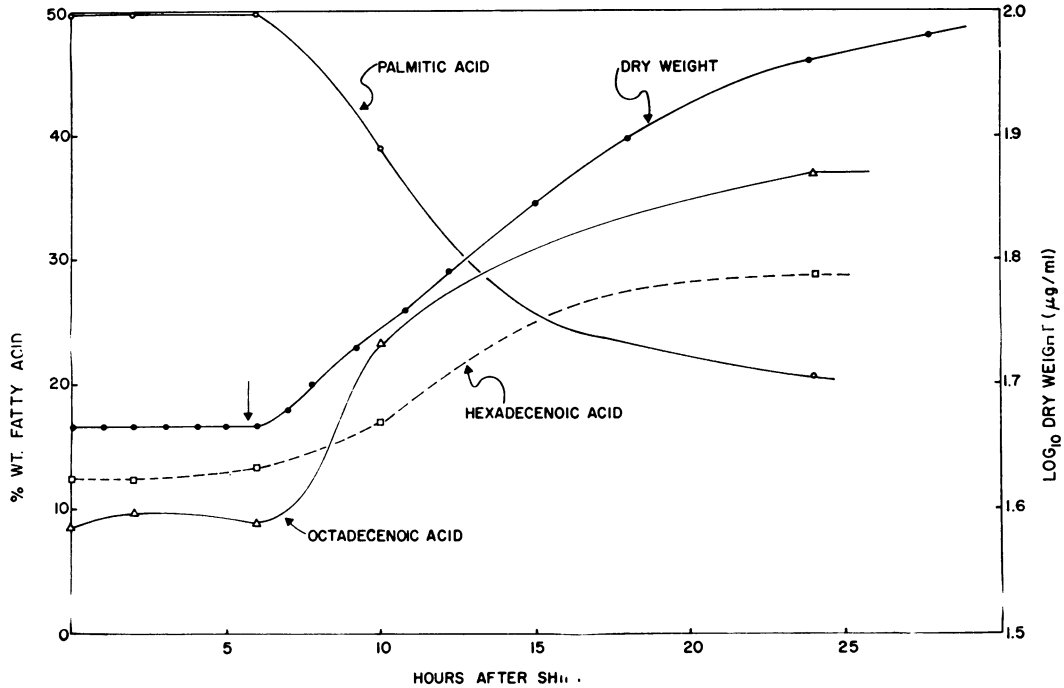

FIG. 4. *Change in dry weight and in proportions of palmitic, hexadecenoic, octadecenoic acids after the shift of a culture of Escherichia coli to 10 C, following a period of 16 hr of starvation for glucose at 37 C. Glucose (0.1%) was added to the culture at the time indicated by the vertical arrow.*

No change in fatty acid composition occurs in the absence of glucose after starvation at 37 C. Significant changes in fatty acid composition commenced only after glucose was added. Under these conditions, growth at 10 C started before the composition of fatty acids had changed significantly; i.e., growth occurred at 10 C in a culture whose lipid composition corresponded closely to that of a culture grown at 37 C.

DISCUSSION

The results show that, on transfer from 37 to 10 C, the lipid composition of *E. coli* becomes less saturated during a 4.5-hr lag period that occurs after the shift. During this lag period, there is no net DNA, RNA, or protein synthesis, no increase in cell numbers, and no increase in cell size. The culture, however, respires at a rate characteristic of a culture growing in the steady state at 10 C (312 μliters of O_2 per mg per hr, Shaw and Ingraham, *unpublished data*).

The change in fatty acid composition of the cellular lipids was the only change observed during the lag period, and it seemed reasonable that this change is prerequisite for growth at low temperature.

To test this hypothesis, we varied environmental conditions to determine whether growth at 10 C always commenced at a specific fatty acid composition. Starvation at 37 C, followed by replenishment of glucose at 10 C at the end of the lag period, allowed disassociation of resumption of growth from change in fatty acid composition. Under these conditions, it was demonstrated that *E. coli* is capable of growing at 10 C with the fatty acid composition it possesses at 37 C.

Marr and Ingraham (1962) suggested that there is a progressive increase in saturated fatty acids and a corresponding decrease in unsaturated fatty acids as the temperature of growth of *E. coli* is increased, but that the fatty acid composition is not directly related to the limits of temperature permitting growth. They based their conclusions on the fact that changes in fatty acid composition could also be achieved by varying the composition of the medium and other environmental conditions.

Kates and Baxter (1962) found that the lipids of psychrophilic yeasts are composed largely of unsaturated fatty acids, regardless of the temperature at which they are grown, whereas the mesophilic yeasts produced unsaturated fatty acids only at low temperatures.

Gaughran (1947), who investigated the lipid composition of steno- and euri-thermophilic bacteria, suggested that the temperature at which the lipids solidify is the minimal temperature for growth, and Heilbrunn (1924) and Bělchrádek (1931) proposed that the temperature at which the lipids melt is the maximal temperature for growth.

Our results are in contradiction of the theory that the minimal temperature for growth is set by the degree of saturation of the fatty acids of the lipids of *E. coli*.

ACKNOWLEDGMENTS

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