JOURNAL OF BACTERIOLOGY, Nov., 1966
Vol. 92, No. 5

Fatty Acid Distribution in Normal and Filamentous *Escherichia coli*

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Received for publication 20 June 1966

Variation in growth medium can induce aberrant morphological forms in *Escherichia coli* B (G. Weinbaum, J. Einstein Med. Cent. **13**:176, 1965). The filaments and branched cells so induced maintain their cell integrity and their ability to divide, though their content of mucopeptide polymer is greatly reduced (G. Weinbaum, J. Gen. Microbiol. **42**:83, 1966). The cell "envelopes" of gram-negative organisms have a high concentration of lipids (M. R. J. Salton, The bacterial cell wall, Elsevier, Publishing Co., Inc., New York, 1964). Therefore, it was of interest to determine whether there is any correlation between lipid composition and these observed morphological changes.

Two strains of *E. coli* were used in these studies. One, *E. coli* B, undergoes morphological change when grown in the inducing medium (IM; see Table 1 for medium composition); the other, *E. coli* E-26, grows normally in IM. Both organisms grow as normal short rods in synthetic minimal-salts-glycerol medium (SM). Cells were obtained from IM or SM media during late logarithmic growth, and were then washed and lyophilized. The fatty acids were isolated by the method of K. Hofmann, D. Henis, and C. Panos (J. Biol. Chem. **228**:329, 1957). Methyl esters were prepared with BF_3 in methanol. Fatty acid analyses were performed by extremely sensitive Golay (capillary) gas chromatography (C. Panos, J. Gas Chromat. **3**:278, 1965). Unsaturated fatty acids were determined after mild hydrogenation (C. Panos, M. Cohen, and G. Fagan, Biochemistry **5**:1461, 1966). The presence of C_{17} and C_{19} cyclopropane containing fatty acids were verified by disappearance after strong hydrogenation with Adams catalyst and glacial acetic acid (R. Kaneshiro and A. Marr, J. Biol. Chem. **236**:2615, 1961). A possibility that the C_{15} acid contains a cyclopropane ring (Table 1) was deduced from relative retention time data, its persistence after mild, but disappearance after strong, hydrogenation, and its comparative behavior with the other two similar acids in these studies. Absolute confirmation, however, must await isolation of a sufficient quantity for degradative chemical procedures. Corrected relative retention times for C_{15} (?), C_{17}, and C_{19} cyclopropane fatty acids (palmitate = 1) were 0.7865, 1.6504, and 3.3371, respectively. Fatty acids were identified by cochromatography with authentic standards when possible. Only fatty acids present in greater than 1% concentrations are recorded. Data are presented as per cent of total fatty acids.

The fatty acid composition of both strains of *E. coli* is presented in Table 1. When *E. coli* E-26 and strain B are grown in synthetic medium, the

TABLE 1. *Fatty acid yield, composition, and ratios of two strains of Escherichia coli*

Fatty acid	*E. coli* E-26		*E. coli* B	
	SM[a] (20.40)[b]	IM[c] (9.38)	SM (6.80)	IM (3.40)
Lauric	3.72	2.84	3.27	6.48
Myristic	6.22	6.68	10.00	9.28
$C_{15}\nabla$ (?)[d]	2.70	2.34	3.84	1.33
Palmitic	45.70	55.30	44.40	54.70
C_{16}:$\Delta^{7,8}$	4.10	3.32	2.27	2.90
Palmitoleic	2.32	1.25	1.05	1.25
$C_{17}\nabla$[d]	16.50	14.20	18.10	5.86
Stearic	1.23	1.99	0.40	1.51
Oleic	1.38	1.46	0.80	2.20
cis-Vaccenic	3.92	1.48	2.68	3.55
Unknown[e]	2.46	1.33	2.13	3.18
$C_{19}\nabla$[d]	9.78	7.65	8.95	4.81
S/U + C[d]	1.46	2.15	1.60	3.43
U/C[d]	0.40	0.31	0.22	0.83
17∇/16:1	2.57	3.11	5.45	1.41

[a] Synthetic medium (grams per liter): glycerol, 4; NaCl, 0.5; $MgSO_4 \cdot 7H_2O$, 0.41; Na_2HPO_4, 6.0; KH_2PO_4, 3.0; NH_4Cl, 1.0. Growth at 36 C.

[b] Numbers in parentheses = per cent fatty acid content.

[c] Inducing medium (grams per liter): Hycase (Sheffield Chemical, Norwich, N.Y.), 50; Nutrient Broth (Difco), 24; glucose, 20; L-lysine, 10; NaCl, 7.6.

[d] Abbreviations: S = saturated, U = unsaturated, C = cyclopropane-containing (∇) fatty acids.

[e] A saturated polar acid; relative retention time (palmitate = 1) = 2.454.

former contains a somewhat higher quantity of unsaturated fatty acids and a lower amount of myristic acid. *E. coli* E-26 has a significantly higher yield of total fatty acids when grown in SM, as compared with *E. coli* B grown in the same medium. Changing the growth medium from SM to IM causes some changes in the fatty acid composition of strain E-26, without an apparent change in cell morphology. The most marked change is the increased amount of palmitic acid. Also, there is a decrease in the unsaturated as well as in the cylcopropane-containing fatty acids. In contrast, J. Karkas, H. Türler, and E. Chargaff (Biochim. Biophys. Acta **111**:96, 1965) found that growth in a complex medium favored the accumulation of cyclopropane fatty acids with a stoichiometric decrease in the corresponding unsaturated fatty acids. It is possible that in strain E-26 we may be observing a feedback inhibition of unsaturated fatty acid biosynthesis leading to decreasing amounts of cyclopropane-containing fatty acids.

The fatty acid composition of *E. coli* B grown in SM is in agreement with that published by others (J. Karkas, H. Türler, and E. Chargaff, Biochim. Biophys. Acta **111**:96, 1965). However, marked changes result in the concentration of these lipids when this organism is grown in IM (Table 1). Although an increase in palmitic acid is noted, as found with E-26, no decrease in unsaturated fatty acids is observed. In fact, an appreciable increase results. The most marked change is the severe inhibition in cyclopropane fatty acid synthesis. The inhibition of these "ring-containing" acids is not environmentally induced (V. Knivett and J. Cullen, Biochem. J. **96**:771, 1965), since it is not observed in strain E-26. Since the increase in unsaturated fatty acids is not stoichiometric with the decrease in cyclopropane fatty acids, we may be observing the same feedback inhibition seen in E-26 as well as an inhibition of methyl transfer for cyclopropane acid formation (H. Zalken, J. Law, and H. Goldfine, J. Biol. Chem. **238**:1242, 1963). Whether this inhibition is at the level of enzyme activity or lack of phospholipid acceptor is presently under study.

There is a correlation between the induction of morphological variants in *E. coli* B and the strong inhibition of cyclopropane fatty acid synthesis. These data are in agreement with others (J. Nesbitt and W. Lennarz, J. Bacteriol. **89**:1020, 1965; M. Kates, Advan. Lipid Res. **2**: 17, 1964), suggesting that cyclopropane fatty acids may play an important structural role in the cell "envelope" of gram-negative organisms.

This investigation was supported by Public Health Service grants AM-O7418, AIO-4495, and AIO-4543, and contract NR 103–576 from the Office of Naval Research. One of us (C. P.) is a Public Health Service Senior Career Development Awardee (7-K3-GM 15,531).