HOMEOVISCOUS ADAPTATION, GROWTH RATE, AND MORPHOGENESIS IN BACTERIA

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ABSTRACT Fluorescence polarization, P, of 1,6-diphenyl-1,3,5-hexatriene was studied in *Escherichia coli* B/r. Modification of nutritional conditions was not compensated by homeoviscous adaptation, demonstrated to exist for temperature variations. Cell diameter, which is known also to vary with nutrition but not with temperature, was found to be positively correlated with $1/P$, and may therefore be regulated by membrane lipid order and fluidity.

INTRODUCTION

Homeoviscous adaptation is a homeostatic mechanism that regulates viscosity of membrane lipids (1) in microorganisms (2), plants (3), poikilotherms (4, 5) and hibernating animals (6) under thermal or pressure (7) stress. In bacteria, many vital processes are intimately associated with the envelope (8) and depend upon the integrity of the cellular cytoplasmic membrane (9). It is therefore conceivable that certain physicochemical properties of the membrane may be maintained at a limited range of values. This has recently been observed for protonmotive force in Gram-negative (10) as well as in Gram-positive (11) species growing under a wide range of pH values or nutritional conditions (12, 13). Homeoviscous adaptation was demonstrated for *Escherichia coli* (*E. coli*) B at various growth temperatures (2), but it took a decade to elucidate the mechanisms underlying this homeostasis (14). Here we show that $1/P$, interpreted in terms of membrane lipid disorder and dynamics (15), is not held constant in bacteria when their growth rate is changed by modifying the medium composition, and is significantly correlated with cellular diameter.

MATERIALS AND METHODS

Strain H266 of *E. coli* B/r (16) was cultivated at 37°C with vigorous shaking. Various growth rates (μ) were achieved in A + B minimal salts solution (17) with the following additions: glucose alone as the sole carbon source or supplemented with 12 or 15 amino acids (17) or with casein hydrolysate (16) (μ = 1.46, 1.82, 2.07, or 2.31 h^{-1}, respectively), glycerol (1.07 h^{-1}), alanine + proline (0.80 h^{-1}), succinate (0.59 h^{-1}), acetate (0.45 h^{-1}) or glucose with glycine replacing ammonia (18) as the sole nitrogen source (0.46 h^{-1}). Fastest growth (μ = 3.0 h^{-1}) was obtained in Luria Broth, and μ = 0 by allowing a lactate-supplemented culture to enter stationary phase. Growth at a wide range of temperatures (25–40°C) took place in minimal-glucose medium (with doubling times of 85–38 min, respectively).

Cells at their logarithmic phase were centrifuged (10 min at 4°C, 12,000 g), washed twice in PBS (phosphate buffered saline, pH 7.4) and pellets were frozen. Pellets were thawed, resuspended in PBS (optical density of 0.25 at 450 nm, measured with Gilford Microsample Spectrophotometer) and labeled (60 min at the growth temperature) in the presence of 3×10^{-5} M DPH (1,6-diphenyl-1,3,5-hexatriene), previously solubilized in THF (tetrahydrofuran) (final concentration 0.1%). Fluorescence (excitation at 360 nm, emission at 430 nm) and its polarization (19) were not changed by washing off excess label; thus, determinations were performed without any wash. (Unlabeled cells were similarly incubated in the presence of THF.) These labeling conditions resulted in steady maximal fluorescence values and minimal scatter contribution (<4%). The outer layers of the bacterial envelope did not present a barrier to the penetration of DPH into the cytoplasmic membrane as was evident from the similar P values obtained after sonicating the cells. The freeze-thaw procedure (see above) did not significantly affect P values and allowed measurements of all samples, prepared in different dates, in a single run.

RESULTS AND DISCUSSION

Heterotrophic bacteria multiply in aqueous salts solutions, at a rate that depends upon the nutrients and temperature (20). A particular medium almost uniquely defines the macromolecular composition of the cytoplasm (20, 21), but changes in membrane structure are superimposed by variation of the growth temperature (2, 14). The influence of nutritional conditions on macromolecular composition is exerted through a multitude of control mechanisms operating by means of soluble effectors (22). The prediction that homeoviscous adaptation is also maintained with regard to medium composition is therefore not self-evident. This prediction has now been tested and indeed refuted (Fig. 1).

Cells of *E. coli* B/r (strain H266, ref. 16) were cultivated at 37°C in different media that support doubling times between 20 and 140 min. Fluorescence polarization P (19, 23), which reflects relative membrane order parameter and microviscosity, is plotted against growth rate (μ, reciprocal of doubling time) in Fig. 1. A systematic decrease in P with μ (~20% over the whole range) is apparent.

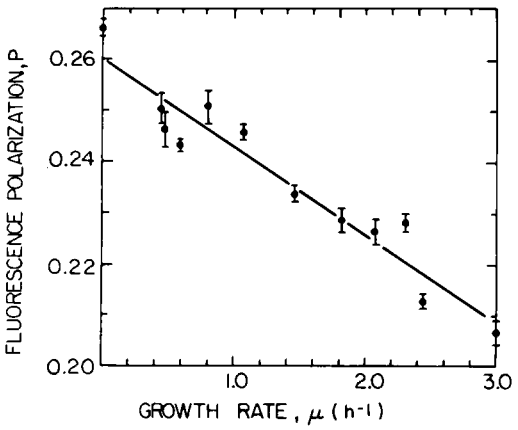

FIGURE 1 Fluorescence polarization P as a function of growth rate. The bars indicate standard errors of the mean P values.

Faster growing cells must have faster uptake rates of building blocks to satisfy their anabolic requirements. However, the surface/volume ratio of such cells is rather smaller (16), and this reduced ratio should therefore be over compensated by an increased number of permeases per unit surface area or by an increased rate of uptake per permease (or both). The latter condition is probably fulfilled: Rates of passive diffusion, facilitated diffusion and protein-mediated transport of glycerol for instance, have all been found to be proportional to membrane fluidity (24).

Faster growing cells are larger (16, 20). At any particular steady-state condition, a bacillary cell extends by elongation and divides at a perpendicular plane. It is therefore surprising that bacteria of certain rod-shaped species are wider at faster growth rates (16, 25). The linear correlation ($r = 0.95$) between $1/P$ and cell diameter (Fig. 2) is consistent with the hypothesis that this physical property of bacterial cytoplasmic membrane is involved in shape determination (8). This hypothesis is concordant with the constant shape observed at varying growth temperatures (26), conditions in which homeoviscous adaptation prevails for the strain studied here (our preliminary, unpublished results) as it does for strain W3102 of *E. coli* (2). This study was carried out with H266 substrains of *E. coli* B/r because its dimensions have been extensively

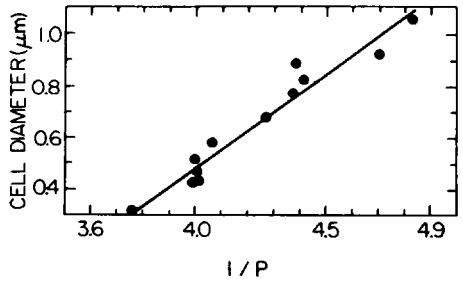

FIGURE 2 Cell diameter as a function of the inverse of P values shown in Fig. 1. Cell diameters were obtained by interpolating the regression line (slope = 0.248, intercept = 0.316) of our published data relating diameter to growth rate (25) for the same strain studied here.

investigated in our laboratory during the last decade (16, 25, 26): most of the range ($0.6 \leq \mu \leq 2.5$) was previously exploited, and for all but four of the media used here it was demonstrated directly that cell diameter is uniquely defined by μ irrespective of medium composition.

The hypothesis of shape determination by membrane fluidity (8), which cannot be ruled out by this report, is also substantiated by the change in cell shape consequent to an artificial modification of membrane fluidity (27) in an unsaturated fatty acid auxotroph of *E. coli* K12. However, the correlations described here between cell dimensions and $1/P$ are circumstantial and only indicative; demonstration of a more direct, mechanistic association requires further experimentation. In addition, eliminating the outer membrane of the cells is important to assure that P determinations pertain to the cytoplasmic membrane, and is thus planned for future research.

Thanks are due to Professors D. Chipman and D. Gill for helpful comments.

This investigation was partially supported by Ben-Gurion University Research Fund (A. Zaritsky) and the Head Scientist, Israel Ministry of Health (A. Parola).

Received for publication 29 November 1984 and in final form 14 March 1985.

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