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BRANCHING OF FAST-GROWING *ESCHERICHIA COLI* 15T$^-$ AT LOW THYMINE CONCENTRATIONS

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1. Introduction

The average size of a bacterial cell growing at a rate μ, and in a steady state, increases exponentially with μ and with the time 'C' taken to replicate the chromosome [1–11].

Despite the regular fashion by which they grow (i.e. by elongation only [8,12]), Gram-negative bacilli in enriched medium are not only longer but also thicker [4,6,9,10,13,14]. Similarly, although specific inhibition of chromosome replication results in filamentation [15], *Escherichia coli* cells increase their volume at slower replication rates essentially by increasing their diameter ([6,14], Meacock, Roberts and Pritchard; personal communication). Longer C periods can be obtained by supplementing Thy$^-$ bacteria with lower concentrations of thymine [16–18]. When various Thy$^-$ strains of *E. coli* are cultivated at relatively fast growth rates ($\mu > 1.2\ h^{-1}$), steady-state conditions cannot be reached and the cells continuously increase in size [6,16,19] even though the cultures are "balanced" [20]. Steady states can be achieved by flooding Thy$^-$, Drm$^-$ cells with deoxyribose-1-phosphate [21–23]. This sugar-phosphate is a breakdown product of deoxynucleotides supplied to the growth medium, is not further degraded in Drm$^-$ mutants, and is required for thymine utilization in Thy$^-$ strains [14,24].

The first series of experiments describing this abnormality was performed with glucose-grown *E. coli* 15T$^-$ strain 555-7 [16]. It was impossible to characterize a final shape because after a long period of undisturbed exponential growth in M9 minimal salts medium ($\mu = 1.5\ h^{-1}$ [25]), the cultures flocculated, preventing further measurements. This obstacle has now been overcome following the recent observation of Kahan (M.Sc. Thesis, Ben Gurion University, 1977) that a slight modification of the salts solution (AB; [26]) lessens the flocculation markedly.

A dramatic, reversible change in the regular bacillary shape of *E. coli* 15T$^-$ is described and the possible mechanisms discussed by which bacterial shape formation may be controlled.

2. Materials and Methods

E. coli 15T$^-$ (strain 555-7 [24]) or its Thy$^+$ transductant [21] was cultivated in AB minimal salts solution [26] supplemented with 0.4% glucose, 50 μg/ml of each of the required amino acids (arginine, methionine and tryptophan), thymine at the concentration indicated and, where stated, with 100 μg/ml of deoxyguanosine. The cultures were vigorously aerated at 37°C in a New-Brunswick gyratory shaker. Lengthening of the cultivation period (to maintain conditions of unrestricted, exponential growth) was achieved by appropriately diluting the culture into fresh, prewarmed medium before it reached absorbance of 0.3 at 450 nm (Gilford microsample spectrophotometer).

Samples were fixed either by 0.25% formaldehyde (for light microscopy) or by 0.1% osmium tetroxide (for electron microscopy). Pictures were photographed under a Zeiss phase microscope. Electron micrographs of cells, air-dried by a modification [10] of the agar-filtration technique [27], were taken at 3600 magnification.

3. Results and Discussion

When *E. coli* 15T$^-$ grow unrestrictedly in minimal salts medium with glucose as the sole carbon source, the gradual increase in average cell size [6,16,19] is accommodated primarily by an increase of cellular girth associated with a change in cell shape from a rod to an ellipsoid form [6]. During longer cultivation under similar conditions with 0.4 μg/ml thymine (a concentration double that sufficient for exponential growth to a density of 10^9 cells/ml [25]), most of the cells lost their uniform shape and dimensions (Figs. 1 and 2) and their surface area became wrinkled and uneven as seen under a scanning electron microscope (unpublished observation). Still more striking was the finding that a small proportion of the population started to branch (Figs. 1 and 2). This transition was not accompanied by any detectable change in the rate of total mass increase (absorbance). It was not possible by simple means to determine whether these branching cells lost viability (as happens during thymine starvation [28]) because they comprised less than 1% of the total population. However, they seemed to constrict occasionally and cast-off smaller cells that were identical to the rest of the population. Moreover, the formation of the branching subpopulation was fully reversible: about 80 min after adding deoxyguanosine to growth medium the culture consisted of smaller (though still irregular) cells (Fig. 3a). The total lack of branching cells after only three mass doublings argues against their death, unless the addition of deoxyguanosine stimulated their disintegration. A series of anomalous cytological changes culminating in sudden disintegration have been observed [29] in *E. coli* K12 cells treated with 5-fluorouracil. This unambiguous death was ascribed to "osmotic imbalance" [30] because it could be reversed by an hyperosmotic milieu. It may be necessary to follow the growth of single cells under the microscope (after restoring the replication velocity by adding deoxyguanosine [21–23]) in order to decide whether these monster cells remain viable and return to their normal, rod shape.

Lower concentrations of thymine metabolites involved with bacterial envelope synthesis [31,32] have recently been proposed (R.H. Pritchard, personal communication) as a possible reason for the increased cellular girth (associated with a decreased in

Fig. 1. *E. coli* 15T$^-$ (555-7) grown exponentially in glucose minimal salts medium supplemented with 0.4 μg thymine per ml about 12 h after dilution from similar growth conditions with 20 μg/ml thymine. × 2000.

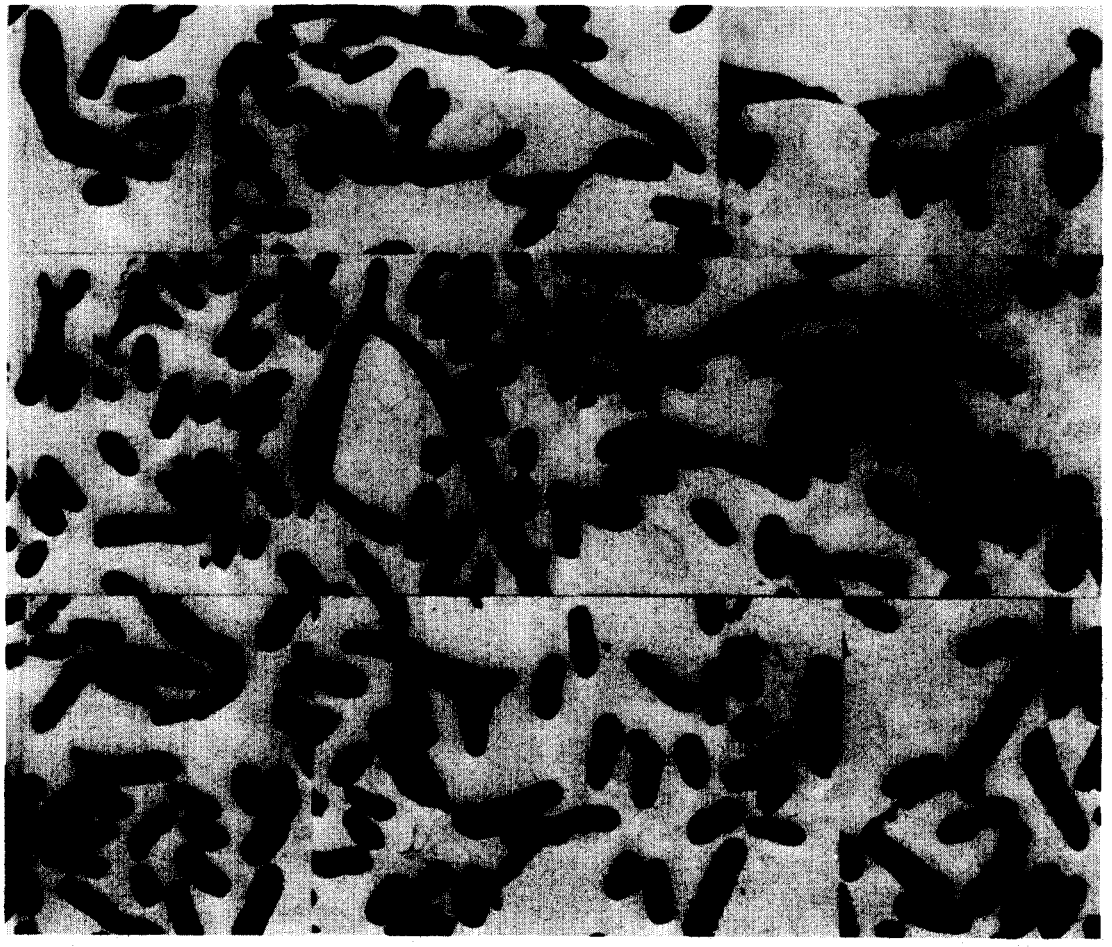

Fig. 2. Electron micrographs of agar-filtered cells of *E. coli* 15T$^-$ cultivated as in Fig. 1.

surface/volume ratio) of Thy$^-$ *E. coli* strains. The observations described here are inconsistent with this view, because branching results in increasing surface/volume ratio. Similar morphological changes have also been observed [33,34] in *E. coli* C and in *E. coli* ML30 under conditions that are not expected to lower the concentrations of the thymine metabolites involved.

A possible explanation for this branching phenomenon is based on the replicon model [35] and on a direct relationship between chromosome replication and cell elongation [6,10,14,36]; a unidirectional mode of replication at a low thymine concentration, observed recently [37] in a Thy$^-$ derivative of *E. coli* K12 (as opposed to the usual, bidirectional replication; e.g. ref. 38), may thus be responsible for this assymetrical mode of growth. The total lack of branching of Thy$^-$ *E. coli* B/r (LEB 16; 18) cultivated under similar conditions (A. Zaritsky and C.L. Woldringh, unpublished observation) may thus mean either that this strain never fails to replicate its chromosome symmetrically or that its envelope is more rigid than that of *E. coli* 15, C and ML30. The latter is favoured in light of the decreased growth rate attained under these conditions in *E. coli* B/r (Woldringh and Zaritsky, unpublished observation). Similarly, lower thymine concentrations increase the doubling time of the Gram-positive *Bacillus subtilis* [17], presumably due to the rigidity of its walls. Distorted cell forms and heterogeneous populations were

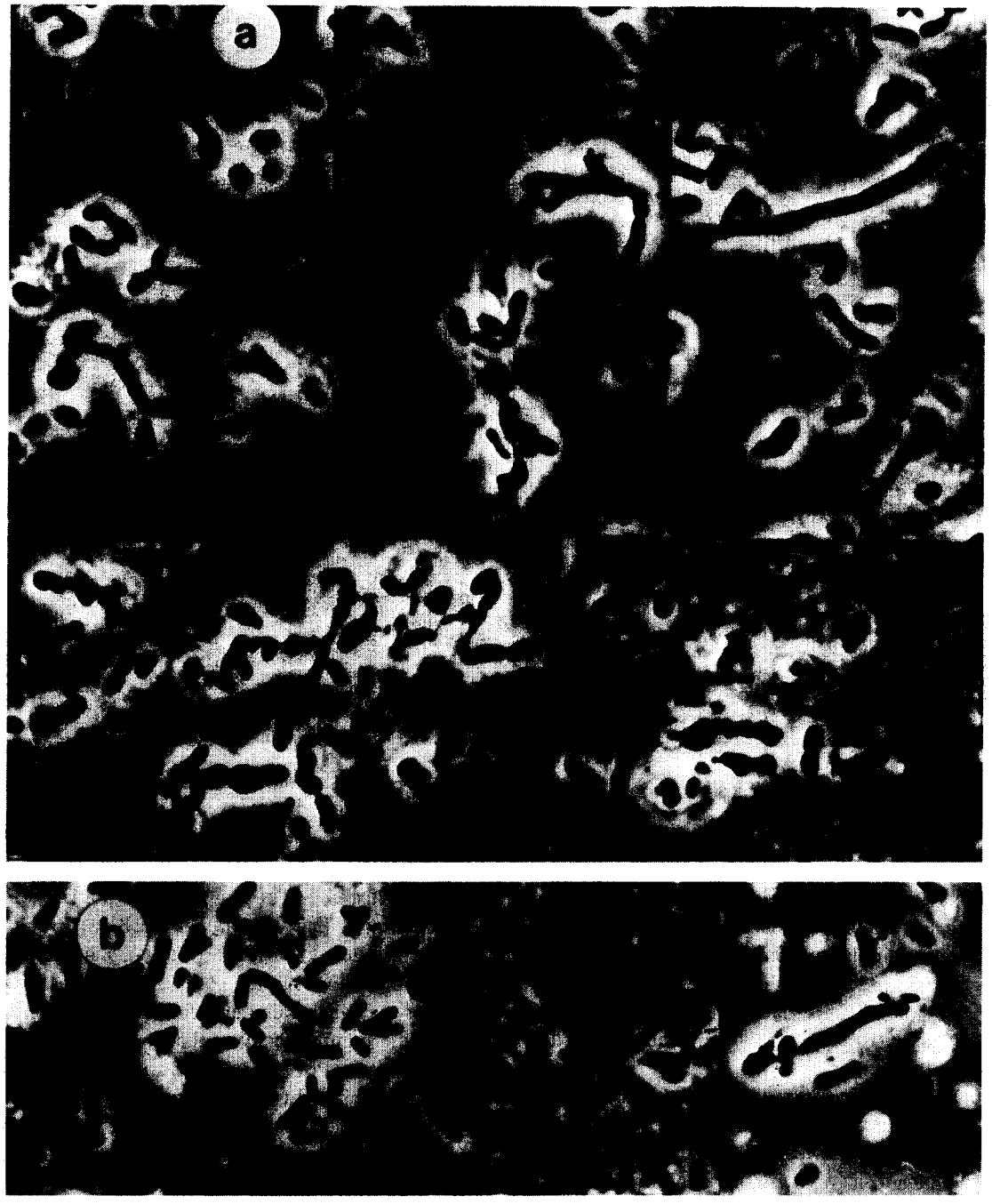

Fig. 3. *E. coli* 15T$^-$ (555-7) grown exponentially in glucose minimal salts medium supplemented with 0.4 μg thymine per ml (a) 80 min after addition of deoxyguanosine and (b) 8 h later. × 2000.

also recorded [39] in an envelope mutant (*envB*) of *E. coli* K12.

Finally, I would like to speculate that the branching phenomenon described here may perhaps be analogous to hyphal branching in filamentous fungi [40], as will be discussed elsewhere (Rosenberger, R.F., Grover, N.B., Zaritsky, A. and Woldringh, C.L.; in preparation).

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