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Relationship of *Escherichia coli* Density to Growth Rate and Cell Age

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The cell densities of *Escherichia coli* strains B/rA, BrF, and K-12 (OV-2) were measured at several growth rates and found to be very near 1.105 g/ml in all cases. Ninety percent of the cells of any exponentially growing population banded at densities differing less than 0.75% from the mean. Synchronized populations of B/rA selected as newborn cells were found to keep their density constant for longer than one generation time. However, if selection was based on cell size, by sedimentation through a sucrose gradient, cell density was found to be almost 2% lower than that of newborn cells, but it reached normal values before the first division had taken place. These results meant that mass and volume during the lifetime of the bacterial cell followed parallel kinetics. It was unlikely that density could regulate any event of the lifetime of a cell; on the contrary, density seemed to be a physical parameter that was well controlled during the bacterial growth.

In recent years several laws to describe the growth of *Escherichia coli* during the lifetime of a cell have been proposed. In some instances, such as elongation measured as either increase in length or increase in outer membrane proteins, the different models are difficult to reconcile with each other (1, 3, 6, 9, 14, 15).

Density is related to mass and volume, and therefore to length and diameter, by algebraic equations. Direct measurements of density in relation to both growth rate and cell age, can then help in the understanding of growth of *E. coli* and in discriminating among the different models. Studies on a strain (OV-25) in which mass increases normally but cell size diminishes as a consequence of an amber mutation in the *wee* gene (10) suggest that density plays an important role in the physiology of the bacterial cell and that cells lose viability when their density increases over a certain value.

We report in this paper our results on the behavior of density relative to growth rate in B/rA, the strain of *E. coli* used for the majority of the physiological work; in B/rF; and in OV-2, a K-12 strain that has been used for genetic work on cell growth and division (5, 10, 16). In addition, we have studied the course followed by density during the lifetime of B/rA cells.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *E. coli* B/rA and B/rF *his thy* were obtained from C. E. Helmstetter; *E. coli* OV-2 has been described previously (6). The cultures were always incubated at 37°C.

Other growth conditions have been described elsewhere (10). Before the start of an experiment, particle increases were followed for at least five doublings after dilution from an overnight culture to check that growth was exponential. The culture media were those described by Donachie et al. (6), as well as M9 with the addition of 0.4% aspartate.

Synchronized cells were selected in all cases by the elution procedure of Helmstetter (7) except in one experiment in which the Mitchison and Vincent sedimentation technique (11) was used. The index of synchrony was calculated as described by O. H. Scherbaum (J. Protozool. 6 (Suppl.):17, 1959).

Cell parameter measurements. Optical density and particle numbers were measured as previously described (10).

The densities of the cells were measured in Ludox HS40 (Du Pont Co.) equilibrium gradients containing polyvinylpyrrolidone (average molecular weight, 40,000), as described by Poole (12). After centrifugation, fractions were collected and the density of each one was calculated as described in reference 10. The viable counts in each fraction were measured by plating a suitable dilution onto nutrient agar plates that were incubated overnight at 37°C.

RESULTS

Relationship of cell density to growth rate. Different growth rates for *E. coli* at 37°C were obtained by changing the culture media as indicated in Materials and Methods. Cells from exponentially growing cultures of B/rA, B/rF, or OV-2 were mixed with Ludox-polyvinylpirrolidone and centrifuged to equilibrium as described by Poole (12). Measurements of the viable count and density of each fraction showed that in all cases 90% of the cells from an exponentially growing population were distributed

within a density band with a width of 1.5% of the mean density value. To assign a density value to a given population, the mean of the density distribution was calculated for each growth rate. These mean values are given in Table 1 for all growth rates studied in the three strains. From Table 1 the mean cell density of each of the populations studied could be calculated. For B/rA, the mean density was 1.105 g/ml (standard deviation, 0.005); for B/rF, it was 1.102 g/ml (standard deviation, 0.002); and for OV-2, it was 1.109 g/ml (standard deviation, 0.006). Taking together all the values obtained for the three strains, the mean density for *E. coli* populations growing exponentially at different growth rates, when measured by sedimentation to equilibrium in Ludox-polyvinylpirrolidone, could be calculated and was 1.105 g/ml (standard deviation, 0.006).

From these results we concluded that the densities of the *E. coli* populations growing at different growth rates fluctuated less than 1% around the mean.

TABLE 1. *Mean density of E. coli cells relative to growth rate*

Strain	Medium[a]	Growth rate (duplications/h)	Mean density (g/ml)
B/rA	10	0.428	1.094
	10	0.631	1.101
	1	0.520	1.102
	1	0.600	1.099
	1	0.600	1.100
	1	0.600	1.108[b]
	1	0.630	1.111[b]
	2	1.140	1.108
	3	1.333	1.099
	3	1.333	1.110[b]
	3	1.500	1.102[c]
	4	1.820	1.106
	5	2.220	1.110
	7	2.730	1.107
	7	2.730	1.112[b]
	8	3.000	1.105
B/rF	2	1.000	1.102
	3	1.330	1.104
	8	2.000	1.101
	8	2.222	1.099
OV-2	10	0.500	1.097
	10	0.226	1.110
	3	0.923	1.114
	4	1.200	1.111
	5	1.714	1.110
	8	2.000	1.114

[a] Media 1 through 8 are described in reference 6; medium 10 was M9 plus 0.4% aspartate.

[b] Mean density of cells synchronized by age selection; the mean densities obtained for all the sampling times were averaged to calculate these data.

[c] Mean density (calculated as in footnote *c*) of cells synchronized by size selection.

Relationship of cell density to cell age. It has been reported that cell density during the lifetime of a cell can fluctuate 6%, with a maximum at birth and division (12). In view of our results, this would mean that the variation of density within one culture would be greater than the total variation found for three different *E. coli* strains. The selection used in those studies to obtain a synchronous population was a sedimentation procedure that is not suitable to select cells of the same age (3). We therefore reexamined the problem, selecting populations of B/rA cells of the same age by the membrane elution procedure (7). The results (Fig. 1) indicated that density fluctuated less than 0.24% around the mean at all cell ages for several growth rates when the selection synchrony was performed based on cell age rather than cell size.

Fluctuation of cell density in populations synchronized by size. To find if selection by size resulted, under our experimental conditions, in a wider variation than that observed for cells of the same age, a sample of small B/rA cells was selected after centrifugation through a linear sucrose gradient, following the procedure described by Mitchison and Vincent (11). The results (Fig. 2) indicated that density after collection from the gradient dropped to a value 2% lower than the density of newborn cells growing at a similar rate (compare with Fig. 1B). The density of these selected small cells increased as they approached division and seemed to remain stable after they had divided.

DISCUSSION

The exact laws that describe the length and surface extension and the mass increase during balanced growth conditions in *E. coli* still are a matter of debate (9, 14, 15). Most of the difficulties in deciding for or against any particular model are technical. The bilinear (or step law) model for elongation is based on step-up experiments (6), and this makes the extrapolation to undisturbed conditions rather questionable. The bilinear model has also been deduced from the Collins and Richmond equation (2) applied to a single generation time (3); this makes its validity over the entire range of growth rates difficult to predict. In contrast, most of the evidence in favor of the exponential mode of extension comes from electron microscopy observations. These produce more precise measurements, but they may cause artifacts at the preparative stage (17).

Density relates to mass and volume by alge-

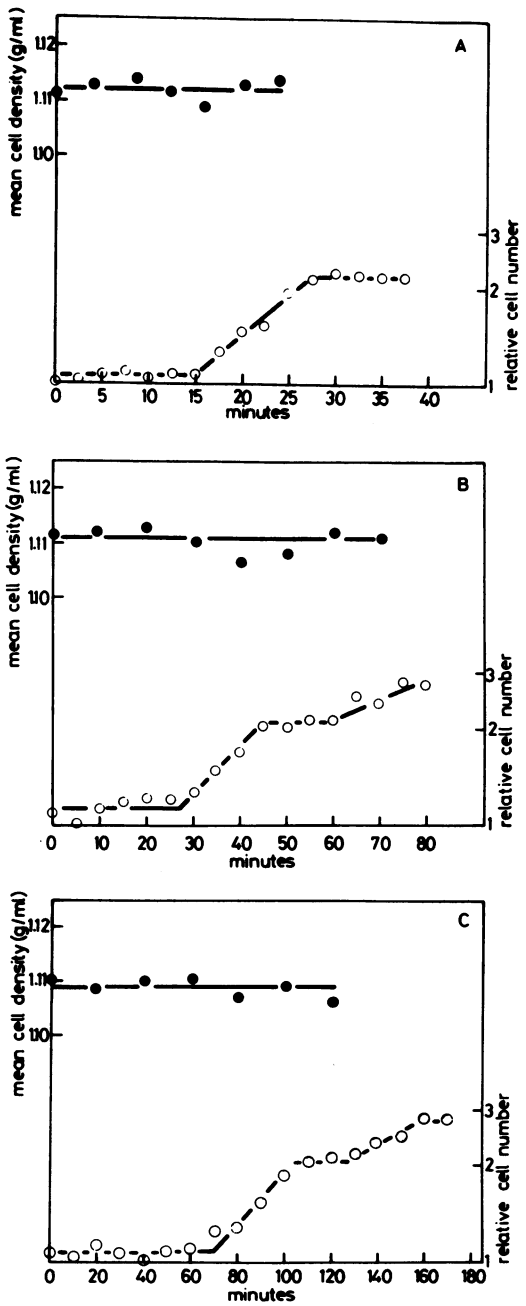

FIG. 1. *Relation of cell density to cell age. E. coli B/rA populations were synchronized by the membrane elution procedure (7). At the times indicated in the graph, 0.5-ml samples were withdrawn to measure the concentration of particles* (○), *and 0.2-ml samples were withdrawn and centrifuged to equilibrium, as described in the text, to measure density* (●). *Three cultures with different growth rates were studied: B/rA growing in medium 7 (6) with a doubling time of 22 min (A); B/rA growing in medium 3 (6) with a doubling time of 45 min (B); and B/rA growing in medium 1 (6) with a doubling time of 100 min (C). Synchrony indexes were 0.68 (A), 0.53 (B), and 0.48 (C). Cell numbers per milliliter at time zero were 1.1 × 10^7 (A), 2.8 × 10^6 (B), and 4.4 × 10^6 (C).*

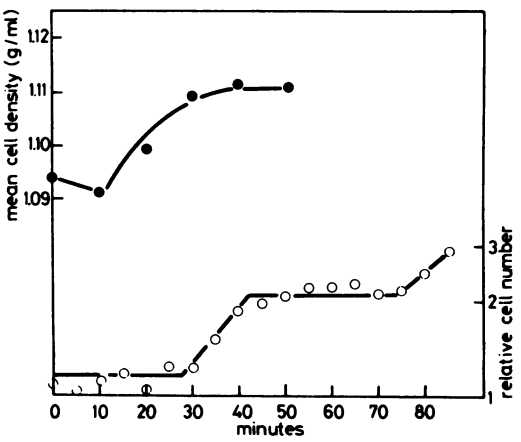

FIG. 2. *Density in a population synchronized by size. The same procedure as that outlined in the legend of Fig. 1 was performed for an E. coli B/rA population of small cells selected after sedimentation through a sucrose gradient (11). The cells were grown in medium 3 (6) with a particle doubling time of 45 min. The synchrony index was found to be 0.53, and the cell number per milliliter at time zero was 2.4 × 10^7. Symbols:* ○, *particle concentration;* ●, *density.*

braic equations, and our data for B/rA indicate that it remains constant within very narrow margins (similar to the variation due to experimental error; compare the results for medium 1 in Table 1) independently of the cell age. Our main conclusion is, then, that increases in mass and volume during the lifetime of a cell should follow parallel courses, regardless of which particular courses they are. Length and mass increases could nevertheless follow different kinetics, provided that diameter changes in a way that compensates for the difference (17).

The mean values of such cell parameters as length, mass, and DNA content, although quite constant for a given growth rate, depend on the growth rate of the culture. This means that dividing *E. coli* cells come in many different kinds. Although initiation of DNA replication has been correlated to the attainment of a mass multiple of the so-called initiation mass (4, 8), other events in the lifetime of the bacterial cell have persistently eluded efforts to be correlated to a physical parameter (8). In this line we find that our results do not support the conclusion of Rosenberger et al. (14) that the rate of elongation increases during the lifetime of the cell coincidently with a decrease in cell density. It is

obvious as well that differences in density cannot be used to obtain populations of cells synchronized by age (12). Moreover, if density and overall concentration of macromolecules were to control any event in the life of a cell, the cell must have an extremely accurate way of measuring differences smaller than 3 parts in 1,000. This was the maximum difference found for cells of the same culture with different ages.

Another possibility, that events during the life of a cell are triggered by the expression of specific genes, such as *ftsA*, seems quite plausible in view of recent reports (5, 16). However, how are these genes switched on and off temporally, or, conversely, how are their products rendered functionally active or inactive during parts of the bacterial cell cycle? These questions are presently unanswered.

It should be noted as well that cell density seems to be almost constant over a wide range of growth rates in B/rA, B/rF, and OV-2 (see Table 1). We have previously observed that genetic alterations which indirectly cause an abnormal increase in density render the cells unable to plate (10). Absolute values for density in that work did not coincide with our present results, since a gradient of a lower resolution power, without polyvinylpirrolidone, was used.

The decrease in density of small cells selected after centrifugation through a sucrose gradient (Fig. 2) could be attributed to changes in the physiology of the cells due to the presence of sucrose (18), in which case it would be an artifact. Another possibility is that cells of less density could possibly migrate towards the bottom of the gradient at a lower speed and, consequently, that the selected population would be formed by cells smaller and of lesser density than the majority of the exponentially growing population (13). Whatever explanation happens to be correct, our results in Fig. 2 suggest that *E. coli* cells are able to correct deviations in their density within the lifetime of a cell. Taken together, all of these observations indicate that homeostasis of the internal medium is kept with an efficiency that is remarkable for an organism considered, at times, as simple as is *E. coli*.

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