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Generality of the Growth Kinetics of the Average Individual Cell in Different Bacterial Populations

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The kinetics of growth of all the cells in a population is reflected in the shape of the size distribution of the population. To ascertain whether the kinetics of growth of the average individual cell is similar for different strains or growth conditions, we compared the shape of normalized size distributions obtained from steady-state populations. Significant differences in the size distributions were found, but these could be ascribed either to the precision achieved at division or to a constriction period which is long relative to the total cell cycle time. The remaining difference is quite small. Thus, without establishing the pattern itself, it is concluded that the basic course of growth is very similar for the various *Escherichia coli* strains examined and probably also for other rod-shaped bacteria. The effects of differences in culture technique (batch or chemostat culture), growth rate, and differences among strains were not found to influence the shape of the size distributions and hence the growth kinetics in a direct manner; small differences were found, but only when the precision at division or the fraction of constricted cells (long constriction period) were different as well.

During steady-state growth a bacterial population grows exponentially in every extensive property. This is true no matter what the pattern of growth and division of the individual cells is. The basic difficulty of determining the precise growth kinetics of individual bacteria is their minuteness; light microscopy lacks enough resolution to visualize bacteria under optimal conditions, and electron microscopy cannot be used to look at living cells. Despite these limitations linear (3, 6, 20, 29; A. Zaritsky, R. F. Rosenberger, J. Naaman, C. L. Woldringh, and N. B. Grover, Comments Mol. Cell. Biophys., in press), exponential (13), and other (9, 17) growth patterns have been suggested for the increase in size of rod-shaped cells of both gram-positive and gram-negative bacteria cultured under various conditions.

Since each pattern implies a particular mechanism for the control of growth it has been suggested that it would apply for bacteria in general (6, 22), and thus it has been assumed to hold for different strains as well as for one strain in a range of growth rates. This implies that for linear models, in which the rate of elongation (6, 8) or surface synthesis (21) doubles at a constant time before cell division, the growth pattern changes with growth rate, whereas for exponential models the growth pattern remains the same.

In the present study we have analyzed the question of generality of the growth pattern without identifying the pattern itself. To this end we have determined length and volume distributions of the cells from 20 exponentially growing populations. As discussed by Koch (12), the shape of a size distribution is determined by (i) the precision of binary fission into two daughter cells, (ii) the kinetics of growth of an individual cell, and (iii) the distribution of the sizes of cells in the final act of division. If the shapes of two size distributions are identical, the average individual cell in both populations must grow and divide in the same fashion. However, for comparison of populations from different strains and culture conditions which differ widely in absolute cell size (8, 23), normalization is necessary. By dividing each cell size by the arithmetic mean size of the sample, cell size (length or volume) becomes a dimensionless parameter, and only the shape of the size distribution or the cumulative size distribution remains to be compared. For comparison of cumulative size distribution we use here the Smirnov-Kolmogorov test (5). This test has been applied routinely in our laboratory to establish constancy of size distributions as a criterion for steady state (13, 26, 28); furthermore, it has the advantage of being nonparametric, so that no assumptions are required with respect to the parameters of the populations.

It was found that there are only small differences among populations of one strain grown in

TABLE 1. Parameters of length distributions[a] of *E. coli* B/r H266 derived from a single clone and cultured at different growth rates

Growth medium	T_D (min)	No. of cells measured	$\bar{L}^b$ (μm)	$2\bar{R}^b$ (μm)	Aspect ratio ($\bar{L}/2\bar{R}$)	Constricted cells	
						%	CV of K(*L*)
Glucose[d]	42	493	2.94	0.74	3.9	16.0	5.8
Alanine + proline[d]	78	852	2.92	0.49	5.9	6.5	7.6
Alanine[d]	135	764	2.73	0.49	5.5	12.8	10.8
Chemostat	104	663	2.47	0.57	4.4	9.7	10.6
Chemostat	166	549	2.43	0.54	4.5	7.5	8.5
Chemostat	416	480	2.14	0.53	4.0	10.2	9.5

[a] For cumulative plots of the normalized distributions see Fig. 2.
[b] $\bar{L}$, $2\bar{R}$, Average length and diameter, respectively.
[c] Coefficient of variation (CV) of the distribution of the ratio between length of each prospective daughter cell and length of the mother cell. This value is higher when the precision of bipartition is lower.
[d] Batch cultures.

either batch or chemostat culture and between different *Escherichia coli* strains. These differences could either be ascribed to the precision achieved at binary fission or to a long constriction period relative to the cell cycle as happens with very fast growing cells. Similarly, the differences between the cumulative size distributions for different bacterial species are small, and the differences could also be ascribed to the two causes mentioned above. We feel that the growth kinetics of the average individual cell may thus be generalized for all species as a working hypothesis until better methods for the determination of bacterial cell size are discovered.

(A preliminary account of this study was presented at the Netherlands Society for Microbiology Meeting at Rotterdam, 1980 [Antonie van Leeuwenhoek J. Microbiol. Serol. 47:181–184, 1981].)

MATERIALS AND METHODS

Bacterial strains and growth conditions. The bacterial strains used are listed in Tables 1 and 2. The *E. coli* B/r strains have been described previously (13, 26). Strain B was obtained from E. Z. Ron, and strain CR34 was from A. Zaritsky. *Salmonella typhimurium* LT2 (1) was grown and fixed by M. Aldea in the same manner as described for *E. coli*. A light microscopy study of the same cultures is to be reported elsewhere (M. Aldea, E. Herrero, and F. J. Trueba, submitted for publication). *Bacillus megaterium* NCTC 10342 was grown in Luria broth (doubling time [T_D] = 19 min) and fixed by I. D. J. Burdett as indicated below for *E. coli*. The bacteria were grown in batch cultures in minimal medium (10) containing different supplements as indicated in Tables 1 and 2. Each culture was started with cells grown overnight on an agar slant, inoculated into 100 ml of medium, and aerated by shaking in a water bath kept at 37°C. Growth was followed by measuring the absorbance at 450 nm with a Gilford microsample spectrophotometer. Exponential mass growth was maintained for at least 10 generations by periodic dilution before sampling for electron microscopy. The steady state of growth was checked by comparing size distributions of samples taken at two different times or by verifying a constant mass/cell ratio for which cell number was determined with a Coulter counter.

E. coli B/r H266 was also grown in a 500-ml Porton-type chemostat (11) in the medium prescribed by Evans et al. (7) for carbon limitation, with glucose as the carbon source. The chemostat was operated at 37°C at pH 7.0 ± 0.1. The dilution rate was varied as required by the experiment. When the culture's dry weight, oxygen consumption, and carbon dioxide production were constant for at least 3 days, the culture was considered to be in a steady state. From each steady-state culture at least two samples were taken for cell size measurements. Dry weight measurements and gas analyses were performed as described elsewhere (19).

Preparation and measurement of cells. Bacteria were fixed with 0.1% OsO_4 and prepared by agar filtration as previously described (28). Electron micrographs were projected on an electronic tablet digitizer (Summagraphics, Fairfield, Conn.), connected to a calculator (Hewlett Packard 9825 T). Lengths and widths of the cells were measured at a final magnification of 10,000 to 15,000× as reported elsewhere (26). From these measurements cell volume was calculated by assuming cell shape to be a cylinder with hemispherical polar caps (21). Because *B. megaterium* cells were twice as big as *E. coli* cells it was sufficient and easier to measure them from phase-contrast light microscope pictures at 7,000× magnification.

Comparison of size distributions. As evident from Tables 1 and 2 the different strains and growth media show great differences in mean size of the population. To normalize the size distributions, measurements within one sample were divided by the sample mean given in Tables 1 and 2. To illustrate the comparison procedure two original length distributions of *E. coli* B/r H266, grown with T_D of 42 and 416 min, respectively, are shown in Fig. 1a. Figure 1b shows the distributions normalized to a mean of unity. Figure 1c is the cumulative plot of Fig. 1b. In this sample the maximal difference between the two cumulative distributions, D_{max}, is 3.7%; the probability that such a difference could occur by chance on the assumption that the two normalized distributions are both random samples from a common distribution is greater than 0.05 (5; D_{max} is called the Smirnov-Kolmogorov statis-

TABLE 2. Parameters of length distributions[a] of different *E. coli* strains grown in batch cultures

Group	Strain	Growth medium	T_D (min)	No. of cells measured	$\bar{L}$ (μm)	Aspect ratio ($\bar{L}/2\bar{R}$)	Constricted cells			
							%	CV of K(*L*)[b]	$T/T_D \times 100$[c]	CV of L_3[d]
1	B/r H266	Alanine + proline	78	852	2.92	5.9	6.5	7.6	9	18.1
	B/r H266	Alanine	135	764	2.73	5.6	12.8	10.8	18	18.7
	B/r K	Alanine + proline	88	597	2.53	4.9	14.4	8.6	19	16.5
	B/r K	Alanine + proline	100	1,873	2.87	5.9	11.7	10.7	17	19.6
2	B/r F		80	495	2.14	3.5	5.7	5.8	4	
	B/r F		165	666	1.69	2.6	11.4	4.9	16	8.0
	B/r A	Alanine + proline	62	1,032	1.92	3.3	12.9	4.7	18	10.6
	B/r A	Alanine	125	1,767	1.66	2.7	9.6	4.2	14	9.3
	B	Acetate	115	420	1.96	2.8	21.4	3.4	28	
	CR34	Aspartate	130	775	2.98	3.9	8.0	5.5	11	
3	B/r H266	Broth	21	1,663	3.81	3.8	34.1	4.5	43	14.8
	B/r K	Glucose + Casamino Acids	27	1,367	2.73	3.6	27.7	5.4	37	11.3
	B/r A	Broth	22.5	1,621	3.12	3.3	43.0	5.4	53	12.2
	CR 34	Broth	19	1,264	3.34	2.9	28.6	4.4	37	21.9

[a] For cumulative plots of the pooled and normalized data see Fig. 3.
[b] See footnote *c* of Table 1.
[c] Relative duration of the constriction period T; CV, coefficient of variation.
[d] Coefficient of variation (CV) of the distribution of L_3, the length of the half of deep-constricted cells; at least 20 deep-constricted cells were measured for each given value.

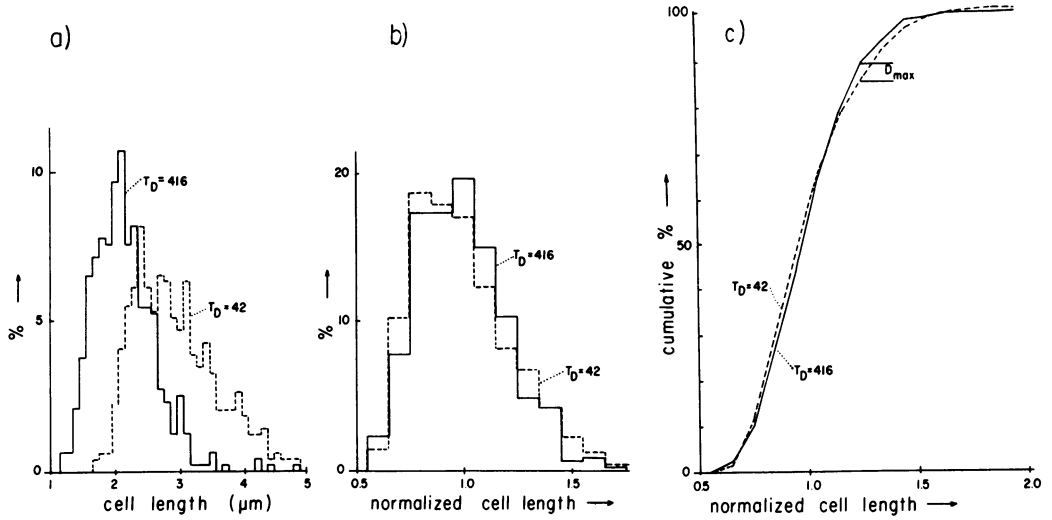

FIG. 1. Length distributions of two steady-state populations of *E. coli* B/r H266. One was grown in a chemostat (T_D = 416 min) the other in batch culture (T_D = 42 min). See Table 1 for distribution parameters and number of cells measured. (a) Before normalization. Note the difference in mean cell length. (b) After normalization; each cell length in (a) was divided by the mean of its distribution, yielding comparable size distributions. (c) Cumulative length distributions of the curves in (b). D_{max}, the Smirnov-Kolmogorov statistic, was 3.7%; D_{crit}, the critical value for D_{max}, was 8.7% (see text for interpretation).

tic). Hence, at the 0.05 level of confidence, which we have maintained throughout this paper, we may conclude for this example that there is no significant difference between the two samples and that the cells of both cultures elongated in the same fashion. As an orientation, the critical value for D_{max} (D_{crit}) for two samples of 500 cells each is 8.6%.

RESULTS

Comparison of one *E. coli* strain cultured at different growth rates. To determine whether the growth kinetics of the average individual cell changes with growth rate, one *E. coli* strain was analyzed at six different doubling times. For this particular growth experiment, performed with *E. coli* B/r H266, the various cultures were inoculated simultaneously with cells from a single clone. In this way it was hoped that possible variations in steady-state properties of the strain caused by different cell histories, would be eliminated. Such non-hereditary variations in, for instance, growth rate have been described by Dennis and Bremer (4) and have also been observed in our laboratory: variations of 10% of the mean T_D have been found (see also reference 27). To extend the range of growth rates to be covered and to compare, in addition, unrestricted versus restricted growth conditions, cells were grown in both batch and chemostat cultures. Parameters of length distributions of the different populations are given in Table 1. In Fig. 2a the cumulative length distributions are compared after normalization. It is clear that the shape of all of the different distributions is similar. The small differences observed in the first part of the curves may be caused by the presence of some 1 to 2% tiny, nucleoplasm-free cells in slow-growing populations (T_D > 100 min) of either batch or chemostat cultures. However, no systematic differences dependent on growth rate could be detected. Moreover, no significant difference appeared between batch and chemostat cultures with respect to either length (Fig. 2b) or volume (Fig. 2c) distributions.

Comparison of different *E. coli* strains. To examine further the generality of the growth kinetics of individual cells, we have analyzed the size distributions of different *E. coli* strains (Table 2). Comparison of the shape of the cumulative distributions by eye did not reveal any obvious differences among strains. We therefore grouped the various distributions according to properties which affect the shape of the distributions such as the precision achieved at division and the relative duration of the constriction or T period as measured in our previous work (13, 28). In this way it was hoped that possible differences of the growth pattern of the populations within the groups would be displayed more clearly.

On the basis of the precision achieved at division (Table 2) and the relative duration of the T period (Table 2), the populations in Table 2 have been divided arbitrarily into the following

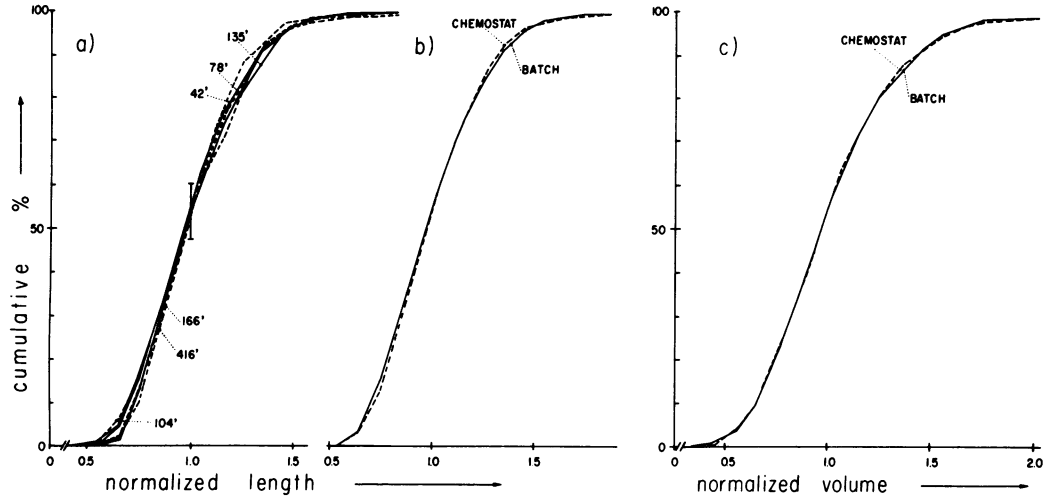

FIG. 2. Normalized size distributions of samples from six exponentially growing cultures of *E. coli* B/r H266. (a) Three batch cultures (——) and three chemostat cultures (-----). The numbers refer to the doubling time of each culture, as listed in Table 1. The vertical segment at length 1.0 indicates the 95% confidence limits for the distribution with the highest number of measured cells (D_{crit} = 5.8%). No curve differs significantly from the others. (b) Same as (a), but after grouping of batch and chemostat cultures. Note the very small difference between the curves. (c) Same as (b), but for volume instead of length.

three groups: (i) slow-growing cells (T_D > 45 min) which divide less precisely (CV of K(L) > 6%; see Table 1 for symbols); (ii) slow-growing cells which divide more precisely about their middle and have a T period shorter than 30% of T_D; and (iii) fast-growing cells, which divide as precisely and have a T period longer than 30% of T_D. The distinction of the more precisely dividing populations into the latter two groups was based on the relatively long T period occurring during fast growth. During this period the growth kinetics can be expected to change, whereas the coefficient of variation of the distribution of dividing cells (Table 2) may increase because of an increased variability in physical separation of the cells.

In spite of the different strains and growth rates occurring in the three groups in Table 2, no significant differences were found between each individual distribution and the distribution derived from the pooled data (D_{max} < 3%; D_{crit} = 3.8 to 6.4%). In other words, for the populations in every group we can detect no differences in the growth course of the average individual cell.

The cumulative distributions derived from the pooled data of the three groups in Table 2 are compared in Fig. 3a and b for length and volume, respectively. Because of the larger number of cells in each of the three distributions (Table 2), significant deviations at the 0.05 level were found for slow-growing cells which divide more precisely (group 2 in Table 2) when compared with the other two groups (see legend to Fig. 3). As to be expected from the higher precision at binary fission and narrow range of the dividing cells in group 2 in Table 2, the deviations only occur in the first part of the distributions, at a size of 0.7 of the mean. We conclude, therefore, that the detected deviation results from a different mode of division and that the growth pattern can be generalized for all three groups.

Comparison with other bacteria. In addition to *E. coli* strains we have analyzed distributions of *S. typhimurium* by the same techniques. The distributions obtained from three different steady-state populations (T_D = 26, 50, and 115 min) were indistinguishable of those obtained from *E. coli* B/r F and B/r A at comparable doubling times (data not shown; D_{max} < 2%, D_{crit} > 4.5%).

A length distribution of *B. subtilis* cells obtained in this laboratory (Fig. 1 in reference 18) appeared not significantly different from the corresponding *E. coli* distribution (group 3) in Table 2. By contrast, comparison of a rapidly growing population of *B. megaterium* (T_D = 19 min) containing 72% constricted cells with the pooled distribution of group 3 in Table 2 showed a significant deviation (data not shown; D_{max} = 9.6%, D_{crit} = 4.6%). Measurements of *Caulobacter crescentus* showed the marked effect of the asymmetrical division of this organism and

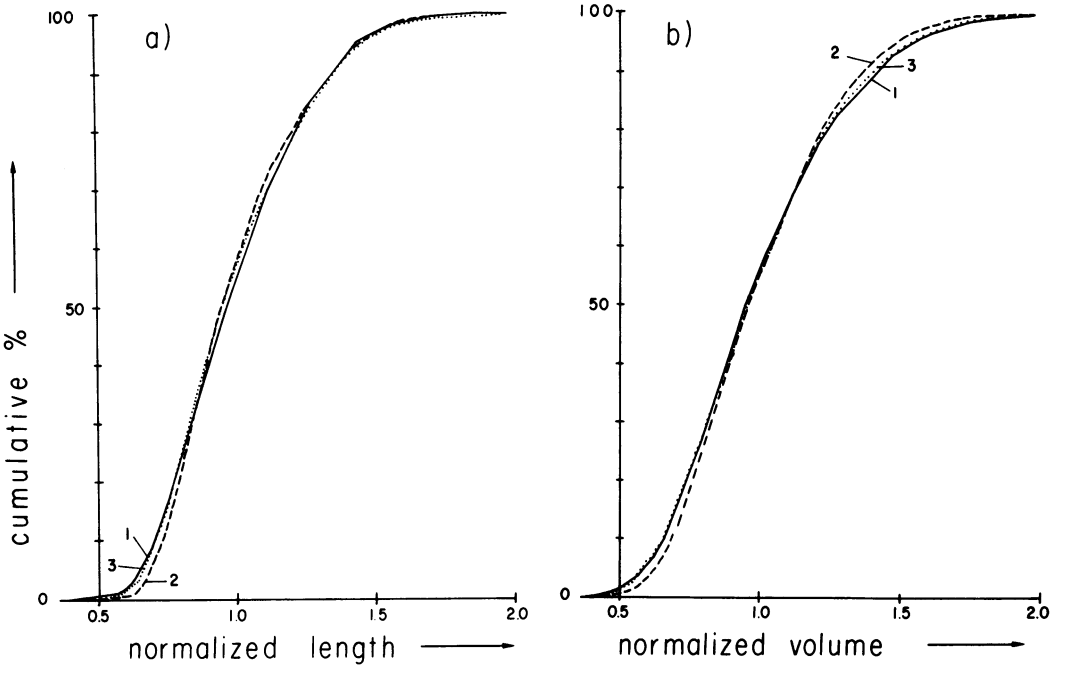

Fig. 3. Cumulative size distributions of normalized and pooled samples from three groups of cultures of *E. coli*. (a) Length and (b) volume, calculated from normalized length and width measurements of individual cells. The numbers 1, 2, and 3 refer to the groups detailed in Table 2. Note that the steepest curve, no. 2, corresponds to slow-growing, precisely dividing cell populations. The maximal deviation of curves 1 and 3 from curve 2 is 3.8%, at size 0.7 (for both length and volume). This is significant by the Smirnov-Kolmogorov test (critical difference: D_{crit} = 2.85% for curve 1 and 2.59% for curve 3).

resulted in significant deviations from the *E. coli* curves of the same magnitude as those of *B. megaterium*.

DISCUSSION

In spite of large differences in size and shape between *E. coli* cells grown at different growth rates (23) (Tables 1 and 2), the mode of growth of the average individual cell as reflected in the shape of cumulative size distributions is very similar (Fig. 2). In addition, the shapes of distributions from various *E. coli* strains (Fig. 3) and even from different bacterial species appeared very much alike.

These results confirm the earlier observations of Kubitschek (14), who analyzed volume distributions measured with a Coulter counter. The theoretical and experimental difficulties inherent to the determination of population distributions with the Coulter counter or by microscopy have been discussed (12, 15). In obtaining our size distributions for microscopic measurement we have tried to reduce the experimental error by starting with a single clone, by growing and measuring cells from both batch and chemostat cultures in a limited period of time and by the simultaneous preparation of all samples for electron microscopy (Fig. 2 and Table 1). In spite of these precautions the shapes of the distributions from a wide range of growth rates did not show significant differences. Moreover, the shape of our distributions very much resembled that obtained from *E. coli* with a flow cytometer (see Fig. 2B in reference 25). With the Coulter counter, however, independent measurements resulted, in our experience, in large differences in shape among the volume distributions of the same populations. Such divergences are also found among published Coulter counter distributions (14, 16, 24).

Differences in imprecision at division and in relative length of the T period could be expected to influence the shape of size distributions (12), and pooling of all of the *E. coli* samples in Table 2 would blur those differences. We, therefore, decided a priori not to pool all samples, but to divide the different populations in three groups. Unexpectedly, no significant deviations between the distributions within those groups could be detected. We therefore pooled the data in the groups and obtained the three curves shown in Fig. 3.

Because no significant difference was ob-

tained between cells of group 1 and 3 in Table 2, the influence of imprecision at division (group 1) and long T period (group 3) appear to be approximately equal for the range of values encountered in *E. coli*. The discrimination of group 2 cells is interpreted to result from the small coefficient of variation of the distribution of dividing cells (Table 2). In spite of this capability of our method to detect small differences between cumulative distributions, no systematic deviations in growth pattern have been found with a change in growth rate. This finding does not seem to agree with linear models in which the absolute growth rate doubles a constant period before or after division (6, 20, 29; Zariksky et al., in press); because the relative duration of this period varies with the doubling time of the culture, a change in growth pattern and thus in the shape of the size distributions can be expected at different growth rates. By contrast the exponential model predicts that the growth curves of the average individual cell in populations with different growth rates will, after a suitable change of scale, be all the same and give identical size distributions.

Whether our measuring is accurate enough to distinguish between an experimental distribution of 500 to 1,000 cells and a theoretical one specified by the various growth models proposed in the literature is currently being investigated (L. J. H. Koppes, I. Naaman, C. L. Woldringh, and N. B. Grover, manuscript in preparation; W. Voorn, F. J. Trueba, and A. L. Koch, manuscript in preparation). Preliminary comparisons of cumulative plots of such theoretical distributions show that, after normalization and for a coefficient of variation of cell size at division of 10%, the D_{max} between linear and exponential growth is 3%. Although this difference may seem small, the cumulative curves in Fig. 3 comprise so many cells (Table 2) that, for instance, the application of the Collins and Richmond's analysis (2; F. J. Trueba, submitted for publication) may now allow for statistical distinction of the underlying growth patterns from those predicted by theoretical models. The present work allows for an extrapolation of the outcome of such an analysis to other strains and to different growth conditions.

In summary, we conclude that, within our experimental and sampling errors, every length or volume distribution of *E. coli* or *S. typhimurium* populations can be regarded as a random sample from one of the standard distributions given in Fig. 3. It appears therefore that different organisms growing under steady-state conditions in a wide range of growth rates and showing similar precision at division exhibit a surprisingly small variation in growth behavior. Techniques more accurate than ours are needed to reveal the differences among them, if they exist.

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