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On Dimensional Determination of Rod-shaped Bacteria

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A formal model which describes quantitatively how the dimensions of rod-shaped bacteria are determined is presented. It is proposed that the rate of extension in length is proportional to the number of chromosome termini and the diameter to the number of chromosome replication positions, and data in the literature are compared with the theoretical predictions made.

1. Introduction

Rod-shaped bacteria extend only in length in a given steady-state of exponential growth (Marr, Harvey & Trentini, 1966). However, faster growing cells are also thicker than cells cultivated in a poorer medium which supports a slower growth rate (μ; Schaechter, Maaløe & Kjeldgaard, 1958). It was also recently documented that increasing the replication time (C) of the chromosome of thymine-requiring strains by lowering the concentration of thymine added to their growth medium (Zaritsky & Pritchard, 1971) also results in an increase in cell diameter, in this case without a distinguishable difference in μ (Zaritsky & Pritchard, 1973). The common denominator to both observations is that an increase in the number of replication positions ($n = C\mu$, Sueoka & Yoshikawa, 1965; Pritchard & Zaritsky, 1970) is associated with an increase of the diameter of the cell. A formal description of shape-determination of *Escherichia coli* and of *Salmonella typhimurium* is made possible by the available measurements of their dimensions under the different growth conditions mentioned above and by several assumptions that have extensive support in the literature.

2. The Shape Factor

The extent of change in average cell length (l) with growth rate (Table 1 column 4) is consistent with the notion that the cell elongates at a number of sites which is proportional to the average number of chromosome termini

TABLE 1

Relative dimensions of S. typhymurium†

μ† (hr^{-1})	V(μgm/10^9 cells)‡ $\log V = 0{\cdot}31\mu + 2{\cdot}14$	d observed† (μm)	l calculated§ $4V/\pi d^2$	$a = l/d$ Calculated§ $4V/\pi d^3$	$a = l/d$ Theoretical‖ $2^{(2D-C)\mu}$
1	2	3	4	5	6
2·73	969·0	1·43	600	419·7	1·0
1·85	502·4	1·22	430	352·5	1·0
1·00	281·8	0·93	400	430·1	1·0
0·61	213·3	0·87	359	412·6	1·0

† Observations are taken from Schaechter *et al.* (1958).

‡ Average cell volume is given in mass units, assuming a constant cell density. The experimental equation relating V to μ is taken from Maaløe & Kjeldgaard (1966).

§ Calculations are based on approximating cellular shape to a cylinder for the sake of simplicity.

‖ Since values are given in relative terms, all constants (k, K, K_2 and K_3, respectively) were normalized. C and D were given the values of 46 and 23 min, respectively (Helmstetter & Cooper, 1968).

The number above each column indicates its ordinal number.

per cell and that the rate of elongation per site is proportional to the cell growth rate (Zaritsky & Pritchard, 1973).
Thus

$$l = K2^{D\mu} \tag{1}$$

where K is a constant and D is the time between termination of chromosome replication and the subsequent cell division (Helmstetter, Cooper, Pierucci & Revelas, 1968).

It has been shown (Donachie, 1968; Pritchard, Barth & Collins, 1969) that existing data are consistent with the hypothesis that initiation of rounds of chromosome replication occurs at a constant mass to chromosome origin ratio and that average cell mass (M) is proportional to $2^{(C+D)\mu}$. If the density of cells does not vary with cultural conditions then M is proportional to average cell volume (V). Thus

$$V = k2^{(C+D)\mu} \tag{2}$$

where k is a constant.

To a first approximation, a rod-shaped bacterium is a cylinder. Since in given growth conditions its diameter (d) remains fixed (Marr *et al.*, 1966) one can express V in geometrical terms

$$V = \pi\left(\frac{d}{2}\right)^2 l. \tag{3}$$

Combining equations (1) and (3) and equating (3) to (2) results in the following:

$$\frac{\pi K}{4} d^2 2^{D\mu} = k2^{(C+D)\mu}, \text{ or } d^2 = \frac{4k}{\pi K} 2^{C\mu}.$$

Thus,

$$d = K_2 2^{C\mu/2} = K_2 2^{n/2} \tag{4}$$

where

$$K_2 = 2\left(\frac{k}{\pi K}\right)^{\frac{1}{2}}.$$

It is convenient to define the shape of a cylinder by means of the ratio between its axes. Thus, a fair approximation of the shape factor (a) of an average cylindrical cell can be obtained by dividing equation (1) by (4) to give:

$$a = l/d = K_3 2^{(2D-C)\mu/2} \tag{5}$$

where

$$K_3 = \frac{K}{2}\left(\frac{\pi K}{k}\right)^{\frac{1}{2}}.$$

Since C and D were shown to have constant values in *E. coli* B/r (growing

TABLE 2

Relative dimensions and calculated values for C and for D in E. coli *15*

Thy† 1	Carbon† source (μ) (hr^{-1}) 2	Thymine† conc. (μgm/ml) 3	Relative† d (measured) 4	Relative† l (calcul.) 5	Relative a (l/d) 6	C (min) calcul‡ 7	C (min) exptl§ 8	D (min)‖ (calcul.) 9
+	Glycerol (1·0)	—	1·00	1·00	1·00	(46)	—	(23)
+	Glucose (1·5)	—	1·16	1·23	1·06	48	—	27
−	Glycerol (1·0)	2·0	1·17	0·78	0·67	73	66	1·5
−	Glycerol (1·0)	0·3	1·41	0·78	0·55	105	102	1·5

† Data are reproduced from Zaritsky & Pritchard (1973).

‡ The value in brackets was assumed (Helmstetter *et al.*, 1968). Other values of C were obtained from relative values of d (column 4) and equation (4) such, that

$$C_x = \frac{[\log (d_x/d_o) + C_o\mu_o/\log 2/2]2}{\mu_x \log 2},$$

where subscript o refers to the reference conditions (first line).

§ Values are taken from Pritchard & Zaritsky (1970, Table 3 and Fig. 2a).

‖ The value in brackets was assumed (Helmstetter *et al.*, 1968). Other values of D were obtained from relative values of l (column 5) and equation (1) such that,

$$D_x = \frac{\log (l_x/l_o) + D_o\mu_o \log 2}{\mu_o \log 2},$$

where subscript o is as in (‡).

The number above each column indicates its ordinal number.

with $\mu \geqslant 0{\cdot}9$) such that $C = 2D$ (Helmstetter *et al.*, 1968) equation (5) can be reduced. Hence, the shape factor, $a = K_3$ and is independent of the growth rate. Table 1 (columns 5 and 6) demonstrates the close agreement of this consequence of the model with the shape factor obtained by calculations based on experimental data with *S. typhimurium*. It should be noted here that C and D in this organism were shown to have very similar values to those found in *E. coli* B/r (Spratt & Rowbury, 1971). Recent electron micrographs taken by Woldringh (1974; pers. comm.) show a remarkable constant shape factor in *E. coli* B/r cells grown at different growth rates ($0{\cdot}6 \leqslant \mu \leqslant 1{\cdot}9$).

It has been found that the increase in average volume of *E. coli* $15T^-$ cells associated with slower replication veolicities (longer C's) is due to their marked thickening (Zaritsky & Pritchard, 1973). Moreover, the data suggest that the larger cells resulting from elongating C are shorter than cells with normal C time (Zaristky & Pritchard, 1973). These findings are in qualitative agreement with the predictions made by the model presented above and are consistent with independent results indicating that D gets shorter when the replication velocity is slowed down by limiting thymine concentrations (Zaritsky & Pritchard, 1973; Pritchard, 1974; Meacock, pers. comm.). Thus, comparing the first two lines in Table 2 demonstrates that the shaped factor is constant also in *E. coli* 15 (thy^+) (column 6) and that C and D do not significantly change with the growth rate (columns 7 and 9 respectively). However, in a thy^- derivative of this strain all three parameters vary as a function of the thymine concentration supplied. The striking resemblance of the values of C calculated from measurements of cellular width (column 7) to those calculated from totally different methods and set of assumptions (column 8) supports the hypothesis that cellular diameter in a steady state of exponential growth of a rod-shaped bacterium is determined by the average number of chromosome replication positions.

3. Discussion

It has been suggested (Donachie & Begg, 1970) that the site where the cell extends its envelope during exponential growth corresponds to the chromosomal replication fork. It this were the case then equation (1) must be substituted either by $l = K2^{D\mu}(2^{C\mu}-1)$ or, if the rate of extension per site is independent of μ, by $l = K2^{D\mu}(2^{C\mu}-1)/\mu$ (Rosenberger, pers. comm.). Similar arithmetical manipulations as done previously show that under this assumption cellular diameter is proportional respectively either to $(1-2^{-C\mu})^{-\frac{1}{2}}$ or to $(1-2^{-C\mu})^{-\frac{1}{2}}\mu^{\frac{1}{2}}$. Both of these predictions are not consistent with the experimental findings (see Tables 1 and 2; Woldringh,

pers. comm.). Further, it can be concluded that the elongation site does not correspond to the chromosomal replication origin. The consequence of this assumption would be a lack of dependence of d on C, while the results (Table 2, column 4) clearly demonstrate the existence of a correlation between these two parameters.

One consequence of the model which was presented before (Zaritsky & Pritchard, 1973) and extended here is that either d or cellular density must vary to a certain degree during the cell cycle, since d is proportional to n and this parameter changes discretely at specific cell ages (Helmstetter *et al.*, 1968). The results recorded in the literature concerning this (Marr *et al.*, 1966) do not show significant variation of cellular width. An experimental procedure to observe small variations in density is sorely lacking at present.

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REFERENCES

DONACHIE, W. D. (1968). *Nature, Lond.* **219,** 1077.
DONACHIE, W. D. & BEGG, K. J. (1970). *Nature, Lond.* **227,** 1220.
HELMSTETTER, C. E., COOPER, S., PIERUCCI, O. & REVELAS, E. (1968). *Cold Spring Harb. Symp. quant. Biol.* **33,** 809.
MAALØE, O. & KJELDGAARD, N. O. (1966). *Control of Macromolecular Synthesis.* New York: Benjamin.
MARR, A. G., HARVEY, R. J. & TRENTINI, W. C. (1966). *J. Bact.* **91,** 2388.
MEACOCK, P. Personal communication.
PRITCHARD, R. H. (1974). *Phil. Trans. R. Soc. Lond.* **B267,** 303.
PRITCHARD, R. H., BARTH, P. T. & COLLINS, J. (1969). *XIX Symp. Soc. Gen. Microbiol. Microbial Growth,* pp. 263–297.
PRITCHARD, R. H. & ZARITSKY, A. (1970). *Nature, Lond.* **226,** 126.
ROSENBERGER, R. F. Personal communication.
SCHAECHTER, M., MAALØE, O. & KJELDGAARD, N. O. (1958). *J. gen. Microbiol.* **19,** 592.
SPRATT, B. G. & ROWBURY, R. J. (1971). *J. gen. Microbiol.* **65,** 305.
SUEOKA, N. & YOSHIKAWA, H. (1965). *Genetics* **52,** 747.
WOLDRINGH, C. L. (1974). Ph.D. Thesis.
WOLDRINGH, C. L. Personal communication.
ZARITSKY, A. & PRITCHARD, R. H. (1971). *J. molec. Biol.* **60,** 65.
ZARITSKY, A. & PRITCHARD, R. H. (1973). *J. Bact.* **114,** 824.