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Surface Growth in Rod-shaped Bacteria

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Various models advanced to explain the relationship between cell dimensions and generation time are compared for rod-shaped bacteria growing under steady-state conditions. Equations are developed for three such models based on the linear extension of surface area. The first assumes that the rate of envelope synthesis is proportional to the instantaneous number of chromosome replication forks per cell; the second, that it is inversely related to the generation time and doubles a fixed time d prior to cell division; the third, that it is constant and doubles at initiation of chromosome replication.

Non-linear least-squares analysis is used to fit the theoretical expressions for mean surface area to values calculated from experimental measurements of length and width by assuming the geometry of a right circular cylinder with hemispherical polar caps. The functions describing area at birth are all discontinuous and cannot be solved by accepted techniques; they can, however, be used to test the internal consistency of each model.

Model 1 is consistent only when lateral extension and septum formation are not considered as independent processes. Model 2 provides a very satisfactory fit, the best estimate for d being 49 ± 4 min. In both cases,

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the values of the parameters obtained are statistically indistinguishable from those predicted on the basis of a much simpler geometry: a circular cylinder with plane parallel ends. Model 3 is unsuitable and can be rejected.

Sources of experimental error and some possible consequences of the simplifications used in constructing the models, are considered. A detailed comparison is made between the control of length extension proposed previously and control of envelope synthesis. The implications of the results are discussed, and a more promising way of discriminating among the remaining models is suggested.

1. Introduction

Rod-shaped bacteria can maintain steady states of exponential growth over a wide range of doubling times. Growth at different rates leads to marked differences in cell dimensions (Schaechter, Maaløe & Kjeldgaard, 1958; Grover, Woldringh, Zaritsky & Rosenberger, 1977) and these variations in length and diameter must reflect the nature of the controls that regulate bacterial envelope synthesis (Previc, 1970; Pritchard, 1974). As an approach to studying such controls, several investigators have compared cell sizes and the synthesis of envelope components during growth under different conditions (Sud & Schaechter, 1964; Zaritsky & Pritchard, 1973; Sargent, 1975; Donachie, Begg & Vicente, 1976; Grover *et al.*, 1977). These studies led to the view that the rate of cell elongation or of envelope synthesis may not be directly proportional to cell mass or to growth rate but, rather, that it may actually increase discretely during the cell cycle (Hoffman, Messer & Schwartz, 1972; Ohki, 1972; Sargent, 1975; Shannon & Rowbury, 1975; Churchward & Holland, 1976; Donachie *et al.*, 1976).

A number of distinct models have been formulated in an attempt to explain bacterial growth, division and shape. Some of them are based on the active control of length extension (Donachie & Begg, 1970; Zaritsky & Pritchard, 1973; Sargent, 1975; Donachie *et al.*, 1976; Grover *et al.*, 1977), others of surface growth (Pritchard, 1974; Pierucci, pers. comm.); all postulate a constant rate of cell elongation that changes concomitantly with specific events in the cell cycle. Suggestions for the particular event have been varied: the termination of chromosome replication (Zaritsky & Pritchard, 1973; Sargent, 1975), the initiation of chromosome replication (Donachie, Jones & Teather, 1973; Pierucci, pers. comm.), the duplication of an unregulated gene (Pritchard, 1974), and the attainment of a specific cell length (Sargent, 1975; Donachie *et al.*, 1976) or a maximal cell density (Rosenberger, Grover, & Woldringh, 1978).

Most of the studies suffer from a dearth of measurements and an absence of rigorous comparison between predicted and experimental values and among

the contending models. In this article we present results of measurements of length and width of *E. coli* B/r at different steady states of growth. The fit of various models to these data and to others (Donachie *et al.*, 1976; Pierucci, pers. comm.) is analyzed, and our inability to reject all the models but one discussed.

2. Theory

Our principal aim in the present paper is to compare the various models advanced to explain bacterial cell growth. For models based on the control of length extension, an adequate theoretical treatment has already been given (Grover *et al.*, 1977); this section deals with surface area extension.

We follow the notation adopted previously (Grover *et al.*, 1977); values at birth (indicated by subscript 0) are obtained by setting the age a to zero in the general expressions and mean values (indicated by a bar above the symbol) by averaging over the generation time τ in the usual way. The symbol k_j represents a proportionality constant in model j.

For exponential volume growth:

$$V = \tfrac{1}{2}V_i 2^{(C+D+a)/\tau}$$

and so

$$V_0 = \tfrac{1}{2}V_i 2^{(C+D)/\tau} \text{ and } \bar{V} = 2(\ln 2)V_0.$$

The equations for the surface area A depend on the particular model used.

Model 1

Envelope is synthesized at a constant rate β proportional to the instantaneous number of growth zones. These zones are formed upon initiation of chromosome replication and continue to act for a period of $E \leq C+D$.

Model 2

The rate of envelope synthesis is inversely proportional to τ and doubles at a fixed time d before division.

Model 3

The rate of envelope synthesis is proportional to the instantaneous number of chromosome origins.

For model 1 we divide τ into 3 intervals by defining two ages a_n, a_m such that $a_n \equiv (n+1)\tau-(C+D)$ and $a_m \equiv (m+1)\tau-F$, where $n \equiv [(C+D)/\tau]$, $m \equiv [F/\tau]$, and $F \equiv C+D-E \geq 0$.

Then for

$a_n \leq a_m$

$$\begin{aligned} A(a) &= k_1 N(n,m)/E, && 0 \leq a \leq a_n \\ &= k_1 N(n+1,m)/E, && a_n \leq a \leq a_m \\ &= k_1 N(n+1,m+1)/E, && a_m \leq a \leq \tau \end{aligned}$$

and for

$$a_m \leq a_n \qquad \begin{aligned} A(a) &= k_1 N(n,m)/E, && 0 \leq a \leq a_m \\ &= k_1 N(n,m+1)/E, && a_m \leq a \leq a_n \\ &= k_1 N(n+1,m+1)/E, && a_n \leq a \leq \tau \end{aligned}$$

where

$$N(n,m) \equiv (2\tau - a_n + a)2^n - (2\tau - a_m + a)2^m.$$

For the other two models

$$\begin{aligned} A(a) &= \beta(\tau + d + a) && \text{for } 0 \leq a \leq (m'+1)\tau - d \\ &= 2\beta(d+a) && \text{for } (m'+1)\tau - d \leq a \leq \tau, \end{aligned}$$

where

$$m' \equiv [d/\tau].$$

Explicit expressions are obtained by substituting $2^{m'}k_2/\tau$ for β and $d - m'\tau$ for d in model 2 and $2^n k_3$ for β and $C + D - n\tau$ for d in model 3.

Mean Areas

Straight-forward averaging of the above expressions gives

$$\bar{A} = \frac{k_1 \tau}{E(\ln 2)} 2^{(C+D)/\tau}(1 - 2^{-E/\tau}) \qquad \text{for model 1}$$

$$\bar{A} = \frac{k_2}{\ln 2} 2^{d/\tau} \qquad \text{for model 2}$$

$$\bar{A} = \frac{k_3 \tau}{\ln 2} 2^{(C+D)/\tau} \qquad \text{for model 3}$$

Initial Values

These are obtained by putting $a = 0$ in the equations for $A(a)$. After re-arranging, we obtain

$$A_0 = (k_1/E)\{2^n(C+D) - 2^m F - [(n-1)2^n - (m-1)2^m]\tau\} \qquad \text{for model 1}$$

$$A_0 = 2^{m'}k_2(1 - m' + d/\tau) \qquad \text{for model 2}$$

$$A_0 = 2^n k_3[(C+D) - (n-1)\tau] \qquad \text{for model 3.}$$

Geometry

In each model, calculations are performed for two idealized geometries, right circular cylinders (radius R, length L) with plane parallel ends and right cylinders (radius R, length $L - 2R$) with hemispherical polar caps. In model 1, two cases are considered: the "open" version, in which sites synthesize lateral envelope but not septa, and the "closed", in which they make both lateral and septal envelope; in models 2 and 3, only the closed version is analyzed. Thus $V = \pi R^2 L$ for the cylinder and $\pi R^2(L - 2R) + (4/3)\pi R^3$ for the hemisphere whereas $A = 2\pi RL$ and $2\pi R(L - 2R)$ in model 1 open and $2\pi RL + 2\pi R^2$ and $2\pi R(L - 2R) + 4\pi R^2$ in the others.

In all cases, it is possible to solve for R (and then L) analytically in terms of A and V. In the open version, the solution for the plane cylinder is immediate whereas for the hemispherical caps a cubic equation in R is obtained with a single real root. In the closed version, both geometries give rise to cubic equations in R with 3 real (and, in general, unequal) roots, only one of which is positive and less than the corresponding value of L.

These considerations, of course, apply equally to $V(a)$ and $A(a)$, to $\bar{V}$ and $\bar{A}$, or to V_0 and A_0.

3. Analysis

Initial guesses for the various parameters were obtained wherever possible by solving appropriate sets of simultaneous equations at two extreme values of τ. These guesses were to be used as starting values in a non-linear least-squares analysis (Marquardt, 1963) based on analytical derivatives. The idea was to divide each τ into a large number of small intervals, calculate $V(a)$ and $A(a)$ for each (the latter, for each model separately), solve for R and L at every point (for both geometries), average over τ, and fit the results to the experimental data. In order to improve the starting values prior to the final analysis, the initial guesses were first introduced into the expressions for $\bar{V}$ and $\bar{A}$ (for each model) and the values of R and L extracted (for both geometries). These were then compared with the measured quantities in an attempt to get a least-squares solution. Unfortunately this procedure did not prove feasible and had to be abandoned. The available data are apparently too scanty and too imprecise to fit with such complex expressions: the solutions do converge after a reasonable number of iterations, but they are unstable.

Our second approach was more successful. We calculated $\bar{A}$ from $\bar{L}$ and R for each geometry and used these to fit with the theoretical expressions of the various models. (Models 2 and 3 can actually be done by linear least squares after suitable transformations.) The equation for $\bar{V}$ is independent of the model, and that was fit the same way. So was V_0, from L_0 and R. [Cell diameter changes only very slightly throughout the cell cycle (Marr, Harvey & Trentini, 1966), so that in effect $R(a) = \bar{R} = R_0$.] All three models yield discontinuous expressions for A_0 which cannot be solved by accepted techniques. They can, however, be used to test the internal consistency of the models by introducing the best estimate of the parameters obtained from the $\bar{A}$ equations into the corresponding A_0 expressions and evaluating the residuals.

4. Results

Mean areas of *E. coli* B/r (strain H266) were calculated from previously published experimental values of $\bar{L}$ and R as a function of τ (Rosenberger

et al., 1978) by assuming cell shape to be a right cylinder with hemispherical polar caps. A non-linear least-squares analysis (Marquardt, 1963) was carried out for each of the models using the 18 points available; all three resulted in excellent fits ($p < 10^{-5}$). However, the estimate of $C+D$ obtained from model 3 ($117{\cdot}1 \pm 5{\cdot}7$ min) is well beyond the accepted range (Helmstetter & Cooper, 1968; Kubitschek & Freedman, 1971; Spratt & Rowbury, 1971; Kubitschek, 1974). Furthermore, a comparison of the slopes (Brownlee, 1965) from the linear regression analysis of model 3 and of $\bar{V}(\tau)$ shows them to be significantly different ($P < 10^{-5}$) even though the latter ($83{\cdot}3 \pm 10{\cdot}7$ min) provides a very good fit to the data ($P < 10^{-4}$). We are thus inclined to reject model 3.

In order to test the consistency of models 1 and 2, we inserted the best estimates of the parameters from $\bar{A}$ into the corresponding expressions for A_0 and compared the values obtained with those calculated from the lengths of newborn cells (Table 1) derived from experimental length distributions as

TABLE 1

Mean length at birth L_0 *of* E. coli B/r *at various doubling times* τ

τ (min)	L_0 (μm)	τ (min)	L_0 (μm)	τ (min)	L_0 (μm)
160	1.625	72	1.750	31	2.417
160	1.500	60	1.750	31	2.292
160	1.442	45	1.900	31	2.233
124	1.875	45	1.833	31	2.067
105	1.708	45	1.833	24	2.533
72	1.783	32	2.200	24	2.025

* *E. coli* B/r strain H266 was grown, fixed and photographed as described previously (Grover *et al.*, 1977). Mean length at birth was estimated from length distribution measurements in the accepted manner (Harvey *et al.*, 1967; Woldringh, 1976).

described previously (Harvey, Marr & Painter, 1967; Woldringh, 1976; Grover *et al.*, 1977). Model 2 provided a very good fit ($P < 10^{-5}$), model 1 did not ($P > 0{\cdot}01$). When the polar caps are added, however, the agreement from model 1 (closed) approaches that of model 2 ($P < 10^{-5}$). The expression for V_0 is not discontinuous, and can be fit to the R, L_0 data in the usual way. The results are very satisfactory ($P < 10^{-5}$) and statistically no different from those predicted by $\bar{V}(\tau)$: $C+D$ is $74{\cdot}4 \pm 8{\cdot}8$ min (s.e.) from $\bar{V}_0$ and $83{\cdot}3 \pm 10{\cdot}7$ min from $\bar{V}$, $\bar{V}_t$ is $0{\cdot}35 \pm 0{\cdot}07$ μm^3 and $0{\cdot}30 \pm 0{\cdot}08$ μm^3.

The behavior of model 1 is interesting. With no restrictions on the parameters, the best fit to the $\bar{A}(\tau)$ data is obtained as $E \to 0$. But the fit remains satisfactory ($P < 10^{-5}$) even when rather severe constraints are imposed, such as holding $C+D$ or $C+D-E$ fixed at some acceptable preset value, and

TABLE 2

Best estimates from $\overline{A}(\tau)$ *for the parameters of model* 1 *(closed) and their standard errors*

Constrained Parameter: $C + D$		
constrained value (min)	estimated parameters E (min)	k_1 (μm^2)
65	34.9 ± 10.0	2.59 ± 0.25
70	48.0 ± 11.0	2.61 ± 0.26
75	62.4 ± 12.3	2.65 ± 0.28
Constrained parameter: $C + D - E$		
constrained value (min)	estimated parameters $C + D$ (min)	k_1 (μm^2)
0	76·4 ± 4·0	2·69 ± 0·24
10	73·2 ± 4·0	2·66 ± 0·24
20	69·8 ± 4·0	2·63 ± 0·23

Every fit is significant beyond the 10^{-5} level.

then E becomes much more reasonable. This is true of both the open and the closed versions; the latter is illustrated in Table 2.

The analysis of model 2 was completely straightforward and yielded estimates of 49·3 ± 3·9 min for d and 1·77 ± 0·16 μm^2 for k_2.

5. Discussion

There are several sources of potential error in the determination of cell dimensions. One of these is the measurement of diameter which, in *E. coli*, can approach the limit of resolution of the light microscope. Electron microscopy has thus been used, but here sample preparation can introduce artifacts (Meacock, Pritchard & Roberts, 1978). This does not appear to be the case with the present technique, however, since our values compare well with those obtained by phase microscopy over the range in which the latter is reliable (Woldringh, unpubl. obs.).

Probably a more fundamental source is the assumption that all cells have identical lifetimes and that each of them divides exactly in half. Both generation time and size at birth are, however, known to be asymmetrically distributed (Powell, 1956). Such asymmetry can be expected to affect L_0 more than $\bar{L}$ (Koch, pers. comm.); the fact that V_0 and $\bar{V}$ provide equally good agreement and with parameters which are statistically indistinguishable, suggests that these simplifications may not in practice have any serious consequences.

Of the various models tested, we have been able to eliminate model 3, because the value of $C+D$ predicted (117 min) is well beyond the range

published in the literature (60–80 min) and inconsistent with that obtained by fitting the same data to expected mean cell volume as a function of growth rate (83 min). The open version of model 1 can also be rejected, on the grounds that it is internally inconsistent: the parameters that best describe the changes in mean surface area with τ provide a totally unsatisfactory fit to the data of surface area at birth as a function of τ. We are thus left with model 2 and the closed version of model 1, and conclude that it is unlikely that lateral extension and septum formation are governed by independent mechanisms.

The shape of *E. coli* is best approximated by a right cylinder with hemispherical polar caps, and that was the shape used. The actual values of the parameters obtained, however, are statistically indistinguishable from those predicted on the basis of a much simpler geometry: a circular cylinder with plane parallel ends. Considerable labor can thus be saved in future by exploiting this empirical fact.

It will be recalled that although model 1 provides satisfactory agreement with the experimental results with reasonable values for the various parameters, the best fit to the data is obtained when E tends to 0. An examination of the expression for mean area as a function of τ shows that as E approaches 0, $\bar{A} \to k_1 2^{F/\tau}$; this is identical (see Theory) to the corresponding expression of model 2, with $k_1 = k_2/\ln 2$ and $F = d$. Thus model 2, which is a special case of model 1, provides the best fit for unconstrained parameters. It should be pointed out that model 1 presupposes a rate of surface extension β that is independent of τ; several observations have been published (Zaritsky & Pritchard, 1973; Sargent, 1975; Donachie *et al.*, 1976; Grover *et al.*, 1977), however, supporting the notion that β is actually inversely proportional to τ, in accordance with model 2.

It has previously been shown (Grover *et al.*, 1977) that quite a different model, one in which the rate of length extension is inversely proportional to τ and doubles once during the life cycle (the "length" equivalent to model 2), suitably describes the functional dependence of L_0 and $\bar{L}$ on τ. Both versions of model 2 satisfy the data equally well ($P < 10^{-5}$), but for completely different values of d: $\bar{A} \propto 2^{4.9/\tau}$ whereas $\bar{L} \propto 2^{1.7/\tau}$. Thus measurements of cell dimensions at different growth rates can only distinguish between the length and surface area versions if d can be determined independently. At present, however, such information is inconsistent. Direct measurements of d, using synchronous cultures, have usually shown that a discrete rate-change does occur (Kubitschek, 1968; Hoffman *et al.*, 1972; Churchward & Holland, 1976; Hakenbeck & Messer, 1977), but they have not been in agreement as to when. This may well be due to the difficulties of obtaining good synchrony without disturbing steady-state growth.

The rate-change in cell extension could occur in response to a specific

signal (Zaritsky & Pritchard, 1973; Pritchard, 1974; Sargent, 1975; Donachie *et al.*, 1976; Grover *et al.*, 1977; Pierucci, pers. comm.) and age τ—d would then coincide with some event in the cell cycle. Both versions, however, generate values for d that can be related to such events: in the elongation version ($d = 17{\cdot}1$ min), it can correspond to the termination of chromosome replication (Zaritsky & Pritchard, 1973; Sargent, 1975; Grover *et al.*, 1977) and in the surface version ($d = 49{\cdot}3$ min), to the doubling of some uncontrolled gene, near the middle of the chromosome, whose product is rate-limiting for envelope synthesis (Pritchard, 1974). It should be pointed out, however, that Donachie *et al.* (1976) have recently disputed the existence of a connection between chromosome termination and d, on the basis of length measurements of thymine-starved Thy^- baby cells selected by sucrose-gradient centrifugation.

While the differences in d predicted by the two models cannot as yet be used to distinguish between them, data from different substrains of *E. coli* may be indicative. Pierucci (pers. comm.) has measured L_0 and R in an *E. coli* B/r *A* substrain and we have compared these parameters and A_0 with our results at different values of τ. The variation in L_0 and R is significantly different in our substrain and in those used by Donachie *et al.* (1976); that in A_0, however, is statistically indistinguishable. Since closely related strains may be expected to have similar values for non-trivial properties, surface area could indeed be the controlled parameter.

Mechanisms other than replication of an uncontrolled gene, may regulate the rate of envelope growth. For example, the number of growth zones could double when the cell attains a constant surface area, in analogy with the constant length (Sargent, 1975; Donachie *et al.*, 1976), or a maximal density (Rosenberger *et al.*, 1978).

Like most workers in the field, we too postulated that cells elongate linearly. This stems in large measure from observations on synchronous cultures (for a comprehensive list of references, see Introduction). In addition, studies on thymine limitation indicated a relationship between elongation rates and termination of chromosome replication (Zaritsky & Pritchard, 1973). Other, less direct considerations also lent support to such models: they accurately predict the observed dependence of cellular dimensions on growth rate (Sargent, 1975; Donachie *et al.*, 1976; Grover *et al.*, 1977; Rosenberger *et al.*, 1978), and new wall appears to be initially laid down in zones (Ryter, Hirota & Schwartz, 1973).

It is, however, very difficult in practice to distinguish between linear and exponential elongation (Kubitschek, 1969); furthermore, current methods of obtaining synchronous cultures are far from perfect. Thus, direct measurements cannot preclude exponential growth, and we now turn briefly to the

possibility that cell extension, like mass, RNA, and protein synthesis, is exponential throughout the life cycle.

Exponential elongation implies a rate of envelope synthesis identical to that of mass during steady-state growth but different during transitions between steady states (Previc, 1970; Pritchard, 1974; Grover *et al.*, 1977). This is no doubt one of the main reasons why exponential growth has rarely been considered in the literature. The recent data of Brunschede, Dove & Bremer (1977), however, do suggest a possible model. Average cellular protein varies with τ differently from average cell mass and follows closely the average DNA. We have shown here (model 1) that average surface area also varies like DNA. But now this is subject to two completely different interpretations. Either $\bar{A}$ is proportional to average DNA because surface area indeed reflects the DNA content of a cell during its life cycle, or it is proportional to average DNA because average DNA is proportional to average total protein and surface area actually reflects the protein content of the cell. The former is the linear model considered above, the latter implies an exponential model and could obtain if a considerable proportion of the many different protein species found in the cell membranes of *E. coli* were required for new envelope synthesis.

All the models discussed above predict quite similar changes in cell dimensions with τ for cultures in steady state growth. They do not, however, predict similar dimensions during transition from one steady-state to another. Analysis of cell dimensions during shifts in growth rate (Sargent, 1975; Donachie *et al.*, 1976) should thus be one way of discriminating among the various models, and we are at present engaged in such studies.

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