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ORIGINAL ARTICLE

When is simple good enough: a comparison of the Gompertz, Baranyi, and three-phase linear models for fitting bacterial growth curves[1]

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The use of primary mathematical models with curve fitting software is dramatically changing quantitative food microbiology. The two most widely used primary growth models are the Baranyi and Gompertz models. A three-phase linear model was developed to determine how well growth curves could be described using a simpler model. The model divides bacterial growth curves into three phases: the lag and stationary phases where the specific growth rate is zero (μ=0), and the exponential phase where the logarithm of the bacterial population increases linearly with time (μ=constant). The model has four parameters: N_o (Log_{10} of initial population density), N_{MAX} (Log_{10} of final population density), t_{LAG} (time when lag phase ends), and t_{MAX} (time when exponential phase ends). A comparison of the linear model was made against the Baranyi andGompertz models, using established growth data for Escherichia coli *0157:H7. The growth curves predicted by the three models showed good agreement. The linear model was more 'robust' than the others, especially when experimental data were minimal. The physiological assumptions underlying the linear model are discussed, with particular emphasis on assuring that the model is consistent with bacterial behavior both as individual cells and as populations. It is proposed that the transitional behavior of bacteria at the end of the lag phase can be explained on the basis of biological variability.*

Introduction

The use of curve-fitting software in conjunction with a primary mathematical model is increasingly being used by food microbiologists to analyze growth data. Currently, the two most widely used mathematical models are the Gompertz equation (Gibson et al. 1988, Buchanan and Phillips 1990, Garthright 1991) and the Baranyi model (Baranyi and Roberts 1994, Baranyi et al. 1995). The former is an empirical sigmoidal relationship, and the latter is a differential equation based in part on the concept that the rate of bacterial growth is controlled by the rate of a 'bottleneck' biochemical reaction. After extensive use of both equations to model a large body of microbiological growth data, we were interested in determining the comparative performance of a simpler model.

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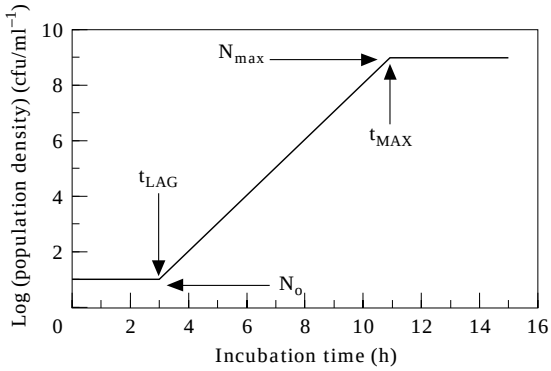

Figure 1. Graphic representation of the three-phase linear model.

Further, we were interested in better defining the physiological basis underpinning growth models, with the goal of developing a conceptual framework that takes into account both the behavior of individual cells and bacterial populations. In particular, there is a need to consider the impact that biological variability has on bacterial growth kinetics. Accordingly, the objectives of this study were to (1) develop a simple linear model that describes bacterial growth curves, (2) provide a physiological framework for the model including assessing the significance of biological variation, and (3) compare the model to the Gompertz and Baranyi models using established growth data for *Escherichia coli* 0157:H7.

Three-phase linear model

The model selected is a three-phase linear one that divides the growth curve into the lag, exponential, and stationary growth phases (Fig. 1). Like the Gompertz and Baranyi models, the three-phase linear model does not consider the death phase. During the lag phase, the cells are assumed to be non-replicating, as they adapt themselves to their new environment. Accordingly, the specific growth rate is zero ($\mu=0$). Once adapted, the cells begin to grow at a rate that is maximal for the microorganism in the specific environment. During the exponential growth phase the specific growth rate is assumed to be a constant ($\mu=k$), with the log of the cell population increasing linearly with time. Once the stationary phase has been reached, there is no net increase in population and the specific growth rate returns to zero ($\mu=0$). The three phases of the model can be described by:

Lag Phase: (1)

For $t \leqq t_{LAG}$,

$N_t=N_o$

Exponential Growth Phase:

For $t_{LAG}<t<t_{MAX}$,

$N_t=N_o+\mu(t-t_{LAG})$

Stationary Phase:

For $t \geq t_{MAX}$,

$N_t=N_{MAX}$

where: N_t=Log of the population density at time t [Log(cfu ml^{-1})]; N_o=Log of the initial population density [Log(cfu ml^{-1})]; N_{MAX}=Log of the maximum population density supported by the environment [Log(cfu ml^{-1})]; t= Elapsed time (h); t_{LAG}=Time when the lag phase ends (h); t_{MAX}=Time when the maximum population density is reached (h); μ= Specific growth rate [Log(cfu ml^{-1})]h^{-1}.

This model provides a mathematical means of fitting growth curves that approximates the way the microbiologists have traditionally estimated growth kinetics graphically. Two-phase linear models of this type have been used in conjunction with curve fitting software to describe microbial growth in food systems (Einarsson 1992, 1994).

Physiological basis for the model

It has been long recognized by a number of microbiologists that not all equations used to describe bacterial growth are models. A concise summary of the requirements for a model was recently provided by Baranyi and Roberts (1995) who pointed out that for an equation to be considered a model and not just a convenient relationship for empirically fitting data, there must be a sound physiological basis underlying the relationship. While the above model is simple in form, it

attempts to take into account the known physiological behavior of bacteria. Further, the model attempts to consider and reconcile the behavior of bacteria both as individual cells and as populations. The following section describes the concepts and assumptions that support the model.

For the purpose of introducing the concepts considered in developing the model, we will use as an example, one of the most studied microbial metabolic processes, the sequential growth of *Escherichia coli* on glucose and lactose as sole carbon sources. When cells are initially grown on glucose and then transferred to a medium that is identical in all attributes except that lactose is substituted as the sole source of energy, the culture enters a lag phase, which is subsequently followed by a re-initiation of exponential growth. During this lag period, the cells adapt themselves to their new environment by inducing the production of lactase, the enzyme needed to hydrolyze lactose. More specifically, the process involves the presence of lactose (and the absence of glucose) inducing the transcription and translation of the *lac* operon, a set of three genes that are coordinately regulated within *E. coli*. Ultimately this results in the cells acquiring the ability to take up and metabolize this alternate carbon source.

From the standpoint of a single cell (Fig. 2), the lag phase can be thought of having two distinct periods. The first period is one of adaptation; the cell senses the need for physiological modifications and expresses alternative metabolic capabilities that allow it to take advantage of its new environment. This period is designated as t_a. In the case of the example, this is the period when the initial lactose molecules are detected by the cell, the *lac* operon is transcribed and translated, and the newly formed β-galactosidase, β-galactoside permease, and β-thiogalactoside acetyltransferase are placed their appropriate cellular sites and activated.

The second period, t_m, is time needed for the metabolic machinery of the cell to generate sufficient energy and then use that energy to produce the array of biological components that are needed for cell replication. Thus, the lag phase is given by:

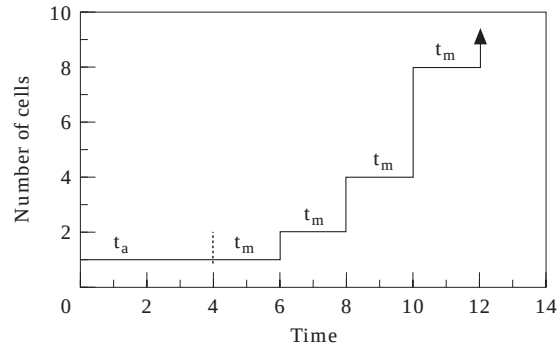

Figure 2. Graphic representation of growth of a single bacterial cell after transfer to a new environment. t_a represents the time needed for the cell to express the new physiological characteristics it needs to function in the new environment. t_m represents the time needed for the cell to generate the energy and biological materials needed to reproduce.

$$t_{LAG}=t_a+t_m \quad (2)$$

Once the original cell divides, the daughter cells, which now have a complete complement of enzymes to utilize lactose, will continue to generate energy and synthesize new cellular material at the same rate. Assuming that no other nutrient becomes limiting, t_m will be constant and equivalent to the doubling rate (and thus proportional to the specific growth rate μ). This implies that t_a and t_m can be estimated from data fitted with the linear model using the relationships:

$$t_m=\text{generation time} \quad (3)$$

$$t_a=t_{LAG}-\text{generation time} \quad (4)$$

The values for t_a and t_m are specific for any combination of culture and environmental conditions. In the case of t_a, the duration of the adaptation period will be dependent on the cell's metabolic status in relation to its new environment, with this being a function of the cell's cultural history. This can be viewed as being equivalent to the 'adjustment function' postulated by Baranyi and Roberts (1994). The more drastic the change in cultural conditions, the more extensive (and likely more time consuming) will be the modifications that the cell has to undergo to adapt

to its new environment. The duration of t_a will also be dependent on the overall metabolic rate of the cell. For example, it could be anticipated that cells transferred from a low temperature environment to a higher temperature would have a shorter t_a than shifting from a high temperature to a lower temperature, even though the differential between the temperatures was the same. This would reflect the fact that transcription and translation of new genes take place more rapidly at the higher temperature.

For t_m, the specific value will be a function of how quickly the cell can generate the energy to carry out the anabolic processes needed for replication and the other factors associated with maintenance of cellular integrity that are competing for that energy. The energy balance within a cell can be defined as:

$$E_T = \Delta E_C - \Delta E_R - \Delta E_H \quad (5)$$

Where: E_T=Overall energy status of the cell; E_C=Energy generated by catabolism; E_R=Energy used to repair damage to the cell; E_H=Energy used to maintain homeostasis within the cell (e.g., pH gradients, osmolarity).

If E_T is positive, then the cell has energy for anabolism and will ultimately replicate. Alternatively, if the balance is negative, then the number of cells will begin to decline. For example, it can be anticipated that increasing the incubation temperature from suboptimal to optimal values would increase overall metabolic rate and thus decrease t_m. Conversely, elevating the incubation temperature above the cell's optimum would increase the amount of energy needed to facilitate cellular repair and thus increase t_m. Similarly, the addition of elevated levels of sodium chloride would require the cell to devote a greater portion of its energy production to pumping Na^+ ions out of the cells and thus extend t_m.

There have been few attempts to rationalize growth kinetics in terms of the known behavior of individual cells. If the replication of a single cell is followed, one encounters a discontinuous function where initially the cell exists for some period of time, followed by a brief transition after which there are two cells. If this process is observed further, the two daughter cells divide after a set period of time, becoming four cells. Accordingly, replication of individual cells can be physiologically described as an exponentially-increasing step function. This can even be observed culturally for a few divisions by inducing a state of synchronous growth.

If the growth of individual cells is a step function, then why do growth curves contain a curvilinear segment during the transition period between the lag and exponential phases? This has been interpreted previously as being a period during which the growth rate increases over time until μ is reached (Buchanan and Cygnarowicz 1990, Baranyi and Roberts 1994). However, it is proposed that this interpretation is inconsistent with the behavior of individual bacterial cells as described above. A transitional μ value implies that the cells are dividing at a rate that is less than their maximum rate. It is difficult to justify a transitional μ value when it is based on only a portion of the population having undergone a single division. We propose an alternate explanation that this transition period actually reflects the biological variation among the individual cells of the bacterial population. Even when working with cultures of isogeneic clones, some degree of variation in the physiological state of the cells must be anticipated. This biological variability must be considered when developing primary models in order to conceptually reconcile microbial behavior as individual cells and as populations.

Returning to the example of the *lac* operon, what would happen to 100 *E. coli* cells that had a t_a=4 h and a t_m=2 h? If there was no variability associated with these values, the cells would be synchronous and 6 hours after being transferred to the lactose containing medium, the 100 cells would abruptly become 200 cells (Fig. 3). However, if a distribution of t_m values among the 100 cells is assumed, then a transition period would be observed (Fig. 3) when the growth of each cell is followed and the number of cells summed. Its similarity to a 'traditional' growth curve becomes even more evident

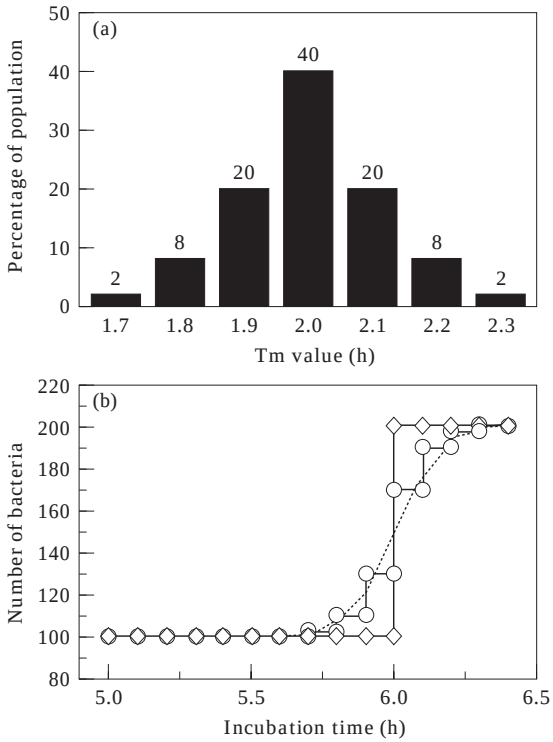

Figure 3. A hypothetical example of the effect that a distribution of t_m values (mean=2) would have on the initial replication of 100 bacterial cells. t_a was held constant at 4 h. The insert depicts the distribution of t_m values that was assumed.
(◇ without biological variation, ○ with biological variation, dotted line represents midpoint population values during replication).

when the population levels are expressed as log numbers.

Assuming that t_m is relatively small compared to t_a, the variances associated with the two segments of the lag phases ($\sigma(t_a)$ and $\sigma(t_m)$) make it unlikely that the second 'step' of replication would be observed experimentally. Instead, it is assumed that there is a smooth transition into the exponential portion of the growth curve. Biological variability also produces a distinct smoothing of the curve during exponential growth. Integration of the individual exponentially-increasing step functions generates the linear relationship between the log of the population density and time that is commonly used to describe μ. This is apparent when the example in Fig. 3 is extended to include the early exponential growth phase (Fig. 4).

The likely impact of variability in t_a and t_m were explored further by performing latin hypercube simulations (McKay et al. 1979) using the program @RISK (Palisade Corp., Newfield, NY, USA). Growth curves were generated by summing the individual step functions for 100 single-cell simulations. The individual step functions were generated using the equation:

$$\text{if } t \leqq t_{LAG}, \text{ the number of cells}=1 \qquad (6)$$

$$\text{if } t > t_{LAG}, \text{ the number of cells}=2^n=2^{(t-tLAG)/tm}$$

where n=number of divisions.

In these hypothetical examples, t_a and t_m were assumed to be normally distributed with mean values of 4 and 1, respectively. The individual contributions of t_a and t_m were evaluated by rerunning the simulations after altering the variance of one term while holding the variance of the second term constant.

When the variance of t_a is small, the transition between the lag and exponential phases is abrupt, while the transition is more gradual when the variance is large (Fig. 5). As would be expected, the growth rate is unaffected since it is a function of t_m. When the variance of t_m was modified while holding t_a constant, there was relatively little impact on the growth curves as long as the variance was small to moderate (Fig. 6). However, when the variance became relatively large, an increase in the growth rates was evident. This represents the likelihood that there are a sufficient number of individual cells with substantially shorter t_m values that would outstrip the growth of the other cells and become the predominant source for most of the population. This behavior is based on the assumption that the variation associated with t_m is related to a heritable characteristic. It can be assumed that there is also non-heritable variation among a population of cells. With non-heritable variation, an alternate assumption is that the variability in t_m for the daughter cells is independent of the t_m value of the parent cell. In that case, the generation-to-generation variation would tend to cancel out and the growth rate (but not the lag phase) would be unaffected by the variance of t_m. It is likely that both situations

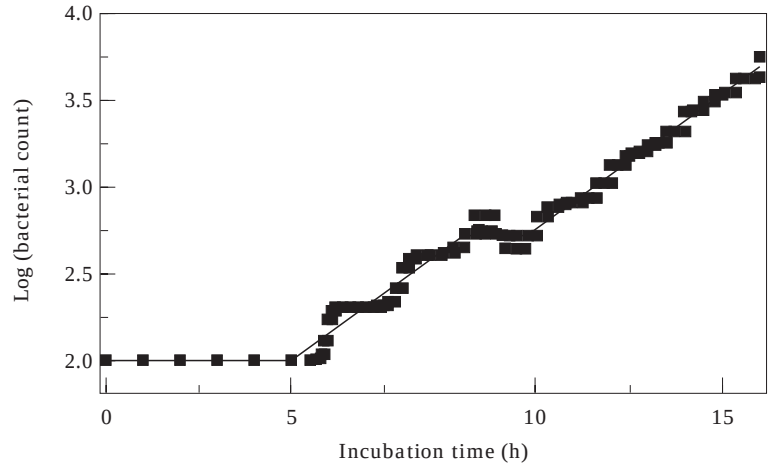

Figure 4. Extension of example depicted in Figure 3 to include a portion of the exponential growth phase.

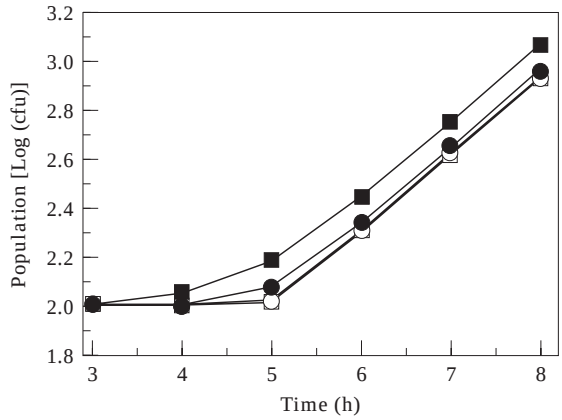

Figure 5. An example of the effect that different variances associated with t_a would have on bacterial growth. Curves represent summation of 100 †*Risk* simulations of individual bacterial cells where t_a (mean=4 h) were assumed to be normally distributed. In all simulations the t_m values was assumed to be normally distributed with a mean of 1 h and a variance of 0·1.
(□ σ=0·01, ○ σ=0·1, ● σ=0·5, ■ σ=1·0).

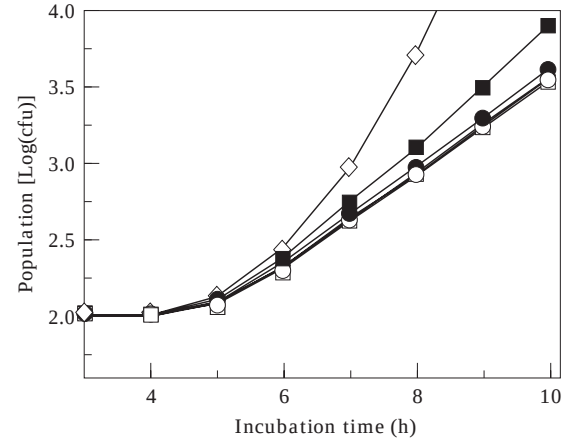

Figure 6. An example of the effect that different variances associated with t_m would have on bacterial growth. Curves represent summation of 100 †*Risk* simulations of individual bacterial cells where t_m (mean=1 h) was assumed to be normally distributed. In all simulations the t_a values were assumed to be normally distributed with a mean of 4 h and a variance of 0·5.
(□ σ=0·01, ○ σ=0·05, ● σ=0·1, ■ σ=0·2, ◇ σ=0·3).

would be encountered in food microbiology. For example, mixtures of bacterial species where there are substantial heritable differences among the species would be expected to produce growth curves similar to those depicted in Fig. 6. However, if one is working with a single strain of a species, heritable differences would be minimal and non-heritable differences will be the major source of t_m variation. In this instance, growth curves similar to those depicted in Fig. 5 would be expected.

The following assumptions were made in proposing the three-phase linear model. Starting with the lag phase, it was assumed that the variances associated with t_a and t_m (and thus the variance for t_{LAG} since $t_{LAG}=t_a+t_m$) are small. This assumption is based on the fact that much of the experimental work

done uses single strains grown under highly controlled, homogeneous conditions. Further, the routine practice of using inocula that have been pre-cultured one or more times in microbiological media further decreases biological variation. Passaging the microorganism in this manner would result in any subclones that had significantly shorter t_a or t_m values rapidly becoming the predominant genotype, thus reducing the variance within the population. The assumption that the variance associated with cell replication is small is supported by the biological variability observed with studies of bacterial cell cycle regulation, though both normal and positively skewed distributions have been reported (Kubitshek 1966, Harvey et al. 1967, Bremer 1982, Trueba et al. 1982, Koch and Higgins 1982, Keasling et al. 1995).

If the variances are small, then the transition between the lag and exponential phases is abrupt and appropriately modeled by the three-phase linear model. It should be noted that if the variability of t_a and t_m were large, there is a distinct likelihood that a single cell would initiate growth well before other members of the population. In this instance, an abrupt transition would be expected because the situation reverts to consideration of a single cell. It is only when t_a and/or t_m have intermediate degrees of biological variability that there would be an extended transition period between the lag and exponential growth phases.

The assumptions that the biological variation associated with t_m is small and normally distributed also affects the growth curve during the exponential growth phase. If N_o is large (10^3 to 10^4 cells ml^{-1}), the exponential growth phase is appropriately described as a linear relationship between the log of the population density and incubation time. As long as all nutrients needed for the generation of energy and the synthesis of new cellular material are in excess, μ will be constant and the exponential growth phase is appropriately described by a linear model.

Ultimately, bacterial numbers reach an upper number which represents the maximum population density that can be supported by the specific cultural environment under consideration. The upper limit is generally in the range of 10^8–10^{10} cfu ml^{-1}, but this is dependent on both the specific environment and species being considered. Typically, as a bacterial culture approaches its NMAX, there is a transition period between the exponential and stationary growth phases when the apparent μ begins to decline. While there have been numerous hypotheses proposed to explain why a bacterial culture has a maximum population density, it is generally accepted that the availability of a limiting nutrient(s) plays an important role (Stanier et al. 1976). We propose that this period represents the time when the assumption that all nutrients are available in excess begins to no longer hold, and the time it takes that nutrient to diffuse to the cell begins to have an effect. This would be most evident when considering the growth of bacteria in a solid matrix, where the cells grow as microcolonies. Ultimately, a point is reached where the rate at which the limiting nutrient diffuses to the microcolony falls to such a value that cells cannot generate or process energy rapidly enough to meet the demands for growth. Then cells would either become dormant or begin to recycle nutrients from cells that have expired. In either case, μ=0.

However, in the three-phase linear model, we have opted to ignore this transition period for two reasons. The first is based on the fact that much of the experimentation done in microbiological modeling uses homogeneous liquid systems. Furthermore, these systems have often been agitated which increases the homogeneity of the environment and ensures that the population grows as individual cells and not microcolonies. In such systems, the need to consider a diffusion term becomes much less important due to the constant mixing of the cultures. Instead, the cultures are more likely to face a situation where there is a threshold concentration below which the cells cease replication. It has been our observation that liquid cultures, particularly when they are agitated, have rather rapid transitions between exponential and stationary growth. The second reason for selecting a simple, abrupt transition between exponential and stationary growth is the

pragmatic realization that most food microbiology applications are not overly interested in the stationary phase. In reality, if the stationary phase is reached, the food is either spoiled if the microorganism is non-pathogenic or a threat to public health if it is a pathogenic species.

Fitting experimental data using three-phase linear, Gompertz, and Baranyi models

The three-phase linear model was evaluated by assessing its fit of experimental data for *Escherichia coli* 0157:H7 (Buchanan and Bagi 1994). The data were also fitted using the Gompertz and Baranyi models, and the growth kinetics derived using the three models were compared. The 18 growth curves (Table 1) used were selected to provide an array of growth conditions and data quality that allowed assessment of the impact of having a variety of growth rates and lag phase durations, varying numbers of data points, and varying distributions of the data points among the three growth phases.

The growth data were fitted to the three models using ABACUS, a curve-fitting program that employs a Gauss–Newton iteration process (Damert 1994). In the case of the three-phase linear model, the parameters fitted were t_{LAG}, t_{MAX}, N_o, and N_{MAX}. The μ value was then calculated using the equation:

$$\mu=(N_{MAX}-N_o)/(t_{MAX}-t_{LAG}) \quad (7)$$

The data were fitted both without fixing any of the models' variables (see below). An exception was the Baranyi M-term which is routinely fixed at 1·00 (Baranyi and Roberts 1995). This reduces the Baranyi model to a five-parameter model, whereas both the Gompertz and three-phase linear models have four-parameters. The goodness of the fit was assessed by determining root mean square (RMS) values:

$$[\Sigma_i(x_{i,calc}-x_{i,experiment})^2/n]^{0·5} \quad (8)$$

where n is the number of data points.

The effect of fixing either the initial population density term or the initial and maximum population density terms was investigated (analyses not shown). This may be necessary with some data sets to get fits that yield realistic growth kinetics values. In general, fixing the variable values had a greater impact on the Gompertz and Baranyi models.

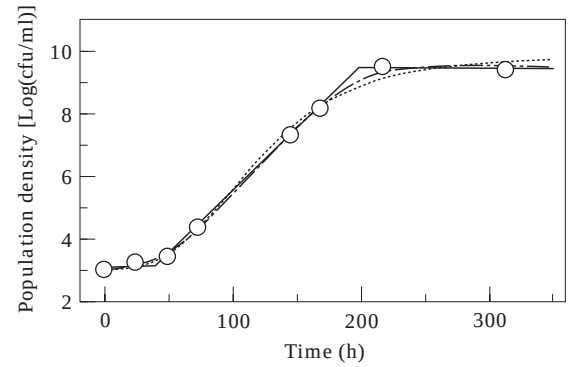

Figure 7. Example of the fits achieved when fitting experimental data using the Baranyi, Gompertz, and three-phase linear models. (—— linear model, - - - - Gompertz model, -·-·-·- Baranyi model).

The growth kinetics and RMS values obtained with the three primary models are summarized in Table 2. An example of typical fits achieved when there is a small to moderate number of data points that are well distributed among the three growth phases is depicted in Fig. 7. In this example all three models fit the experimental data well, with the largest differences being observed during the transition period between the exponential and stationary growth phases. Comparison of the RMS values (Table 2) indicated that none of the models consistently provided the 'best' fit for all the cultures. Overall, the Gompertz model tended to have the highest RMS values, whereas the Baranyi model tended to have the lowest values. The differences in RMS values between the linear and Baranyi models were small. Neither the Baranyi nor the Gompertz model would fit the data for culture 4, and the Baranyi model would not fit cultures 13 and 14. Also, the *v*-values for the Baranyi model had to be fixed before it would fit the data for cultures 3, 7, 9, 15, and 16. Personal experience with the Baranyi model with curve-fitting software has shown that the model is sensitive to the number of

data points and their distribution. In such instances, the curve-fitting routine employed would not converge unless a pair of *v* or q values that provided reasonable growth kinetics values were estimated and either the *v* or q value was fixed. This sensitivity appears to be reduced by a recently proposed reparameterization of the model (Baranyi et al. 1995). As might be expected from a simpler model, the three-phase linear model proved to be more 'robust' than the other models when used with ABACUS. It had distinct advantages compared to the other models when the data set had a limited number of data points or when the data points were not distributed evenly among the three growth phases. Overall, the performance of the three-phase linear model was comparable to the other two primary models.

While growth kinetics obtained with each of the models were similar, there were some systematic differences among the models. The linear model consistently gave lag phase duration values that were shorter than the other models. While the values provided by the three models are similar, they are mathematically describing different things (Garthright 1991, Baranyi and Roberts 1994). In the case of the three-phase linear model, the lag-phase duration can be considered the mean time it takes a population of bacterial cells to undergo their first division. Considering that there is no generally accepted quantitative definition for the boundary between the lag and exponential growth phases, that assumed by the linear model appears reasonable and justifiable.

The generation times obtained from the *E. coli* data sets using the three-phase linear model were on average 22 and 32% greater than values of the Gompertz and Baranyi models, respectively. This reflects the fact that the linear model assumes that the growth rate is constant over the course of the exponential growth phase. With the Gompertz model, the growth rate changes with time, and the μ is described using the maximum value that is associated with the sigmoidal curve's inflection point (Garthright 1991). While the Baranyi model approaches a linear relation, unless it is assumed that μ is a constant, the specific growth rate μ_{max} is the slope through the curve's inflection point (Baranyi and Roberts 1994).

The three-phase linear and Baranyi models predicted similar maximum population densities. These values were typically smaller than the values provided by the Gompertz model (Table 2). The Gompertz model tends to overestimate the maximum population density, particularly when the number of data points during the stationary phase is limited. Like the Baranyi model (Baranyi and Roberts 1995), the linear model can be used effectively in the absence of stationary phase data. In this case a reasonable value for N_{max} is inputted and fixed. Since μ is constant, different combinations of N_{max} and t_{max} will not affect the values derived for t_L or μ.

Conclusions and implications for future research

The three-phase linear model proved to be a simple, robust primary model that compared well with established models. It gave growth kinetics values that were similar to those derived using the Gompertz and Baranyi models, and the 'goodness of fits' of the three models were similar. Its simplicity and flexibility appears to offer a number of advantages, when the assumptions underlying the model (e.g. variances associated with microbial population are small) are valid for the growth data being considered.

The model was developed on the basis of known physiological and culture behavior and offers a conceptual framework around which this and other models can be assessed. The model introduces two factors, the importance of accounting for biological variation and the subdivision of the lag period into two periods, that help reconcile the known behavior of bacteria as individual cells and as populations. It should be possible to evaluate experimentally the significance of both hypotheses. In the case of biological variation, it is possible to estimate the variance of t_{LAG} and μ by direct observation of individual cells. In fact, Kelly and Rahn (1932) microscopically observed the growth of individual cells of several bacteria and found growth rates to be normally distributed, with the

Table 1. *Escherichia coli* 0157:H7 growth data fitted by the three-phase linear, Gompertz, and Baranyi models

Time (h)	Culture Number																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
0	4·10*	2·98	3·10	3·07	3·14	4·26	3·25	3·04	2·68	3·19	3·02	3·13	2·94	3·19	3·12	3·99	2·93	3·19
1						4·17			2·99	3·22		3·18						
2							2·98	3·13	3·83		3·76	3·61						
3					3·16	4·16			4·34	4·58		4·71						
4							3·44	3·73	5·38		5·21	5·53						
5						4·53			6·21	5·51		6·81						
6					3·58		4·14	4·84	6·59		7·28	7·54						
7						4·51			8·22			8·77						
8								5·62	8·68	8·26	8·52	9·14						
22																4·16		
24	4·29	3·24			6·70	7·56	9·49					9·26	4·63	3·33	3·90		3·24	3·92
25								9·28		9·25	9·17							
27					7·17	8·18	9·51											
28									9·58	9·49								
29														3·40				
30					7·66	8·45	9·61	9·34			8·80	9·43	4·73					
31										9·49								
32									9·35									
46						9·56												
48	5·12	3·46	4·40	3·40	9·63		9·70						5·77		4·87		3·91	4·75
50														3·25		4·02		
51							9·72											
52					9·70													
54					9·70	9·56	9·96						6·22	3·37				
70																4·27		
72	6·18	4·37											8·69		5·62		4·13	
75													9·05					
78													9·78					
96													9·92		6·88		4·35	7·44
99															7·30		4·69	7·62
102																	4·77	
103															7·40			7·39
120														4·59				
144	9·55	7·39	8·66	4·12										4·52				
168	9·92	8·20	8·89											4·81	9·92	4·49	7·26	9·68
172																	7·20	
174															10·13			9·75

Table 1 (continued).

Time (h)	Culture Number																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
190																	7·56	
192			9·65	3·97										5·68	9·84			9·35
216		9·53		3·96										6·77				
217																	8·12	
222				4·00														
240																4·92		
241																	8·70	
265																	9·12	
288				4·46														
312		9·45		4·72												5·07		
315														8·08				
360				5·98														
384				5·56														
390				5·50														
408																5·18		
456				8·04														
462				7·92														
482				7·24														
552																5·18		

*Log(cfu ml^{-1})

Table 2. The growth kinetics and root mean square values obtained with the three primary models

Culture Number	Temp. (C)	pH	NaCl (%)	$NaNO_2$ (μg/ml)	Generation time (h)			Lag phase duration (h)			Maximum population Density [Log(cfu ml^{-1})]			Root mean square		
					Linear	Gompertz	Baranyi	Linear	Gompertz	Baranyi	Linear	Gompertz	Baranyi	Linear	Gompertz	Baranyi
1	10	5·5	0·5	0	6·8	5·4	5·1	22·9	33·1	36·3	9·9	10·8	10·1	0·086	0·063	0·036
2	10	6·5	0·5	0	7·5	6·1	6·3	39·8	47·9	51·3	9·5	9·8	9·6	0·080	0·175	0·102
3	12	6·5	2·0	0	7·6	6·0	6·5	12·9	20·0	19·5	9·7	10·4	9·8	0·231	0·129	0·149*
4	12	6·5	3·5	0	15·4	—**	—	263·0	—	—	7·6	—	—	0·412	—	—
5	19	6·5	0·5	0	1·8	1·5	1·7	3·5	5·4	3·6	9·7	10·3	9·9	0·025	0·199	0·027
6	19	7·2	0·5	0	1·7	1·5	1·5	5·6	7·1	6·6	9·6	9·8	9·6	0·104	0·077	0·070
7	28	7·0	3·5	0	1·0	0·6	0·6	2·8	4·3	2·6	9·7	9·8	9·3	0·124	0·110	0·063*
8	28	7·5	5·0	0	0·6	0·6	0·6	2·5	2·6	2·5	9·3	9·4	9·3	0·059	0·082	0·053
9	37	6·5	0·5	0	0·4	0·3	0·3	1·0	1·4	1·4	9·5	9·6	9·5	0·202	0·258	0·199*
10	37	6·5	0·5	100	0·4	0·3	0·3	1·5	1·9	2·3	9·4	9·4	9·0	0·168	0·226	0·107
11	42	8·5	0·5	0	0·4	0·3	0·3	1·1	2·0	2·3	9·0	9·0	9·0	0·162	0·168	0·107
12	42	6·5	0·5	100	0·3	0·2	0·3	1·6	2·0	1·9	9·3	9·5	9·4	0·100	0·190	0·122
13	19	8·0	3·5	0	3·2	3·0	—	12·3	16·0	—	9·9	13·1	—	0·409	0·430	—
14	19	4·5	2·0	0	13·1	12·4	—	82·5	87·8	—	8·1	9·5	—	0·241	0·229	—
15	12	5·5	0·5	0	6·8	6·0	6·3	8·9	15·0	13·8	10·0	11·4	10·4	0·142	0·204	0·190*
16	10	4·5	0·5	0	77·4	61·9	36·7	44·3	61·9	53·5	5·2	5·2	5·3	0·069	0·071	0·079*
17	12	7·5	0·5	200	11·1	9·1	7·6	23·4	48·2	69·0	9·1	10·3	9·2	0·267	0·178	0·226
18	12	6·0	0·5	0	6·2	5·4	5·0	11·2	17·0	25·8	9·6	10·2	9·7	0·165	0·196	0·170

*The *v*-value for the Baranyi model had to be fixed before the data yielded realistic growth kinetics.
**Could not get model to provide realistic fits with these data.

growth rate of the daughter cell being independent of the parent. This technique and other means of segregating cells on the basis of size have been used since to study the relationship between cell size and generation times. An alternate approach to determine variances in μ is the use of cultures that have been diluted to contain a single cell.

Unlike the Gompertz and Baranyi models (but like the reparameterization of the Gompertz model by Zwietering et al. (1990)), the three-phase linear model has a specific term for t_{LAG}. This offers distinct advantages in terms of accounting for the effect of culture history on this period of adaption. As functions are identified that describe for the effect a cell's previous environment has on this growth parameter, they can be readily substituted into the model, i.e., $t_{LAG}=F(x)$. For example, it is possible that the adjustment function proposed by the Baranyi model can be interpreted as the distribution of lag times. The systematic differences observed in predicted lag time values with the three models highlight the fact that there is currently no generally accepted definition for lag phase that is based on physiological events occurring in the bacterial population. The separation of the lag phase into an adjustment period and a metabolic period should allow this process to be better evaluated experimentally. For example, the *lac* operon could be to estimate t_a and its variance for the glucose to lactose transition by determining the time it takes cells to begin expressing β-galactosidase. The t_m portion of t_{LAG} also has implications for future research. One of the most obvious of these is that this offers an explanation for the apparent correlation between specific growth rates and lag phase durations that has been observed previously by a number of investigators (Smith 1985, Griffiths and Phillips 1988, Mackey and Kerridge 1988). This relationship becomes more understandable when a segment of the lag phase is equivalent to the doubling time of the cell. The proposed relationship between t_m and the energy status of the cell may offer new avenues for relating the growth of the cell to its physiological status, and thus lead to the development of more mechanistic models for describing the behavior of microorganisms in foods. An immediate goal of predictive food microbiology should be to explore how the primary models currently being used can be conceptually integrated with the models that are being developed by bacterial physiologists to describe cell cycle regulation (Keasling et al. 1995).

The summary, the three-phase linear model appears to be a simple, effective primary model that can be used readily with curve fitting software to estimate bacterial growth kinetics. Further, the model advances the goal of developing more physiologically based models by introducing the importance of both considering biological variability and establishing the need to reconcile the growth characteristics of bacterial populations with the known behavior of individual cells. We are currently exploring the development of a more sophisticated version of the model that includes appropriate terms for the variances, thereby more accurately describing the transition between lag and exponential growth phases.

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