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A new logistic model for *Escherichia coli* growth at constant and dynamic temperatures

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Abstract

A new logistic model for bacterial growth was developed in this study. The model, which is based on the logistic model, contains an additional term for expression of the very low rate of growth during a lag phase, in its differential equation. The model successfully described sigmoidal growth curves of *Escherichia coli* at various initial cell concentrations and constant temperatures. The model predicted well the bacterial growth curves, similar to the Baranyi model and better than the modified Gompertz model, especially in terms of the rate constant and the lag period of the growth curves. Using the experimental data obtained at the constant temperatures, the new logistic model was studied for growth prediction at a dynamic temperature. The model accurately described *E. coli* growth curves at various patterns of dynamic temperature. It also well described other bacterial growth curves reported by other investigators. These results showed that this model could be a useful tool for bacterial growth prediction from the temperature history of a tested food.

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1. Introduction

Recently, a number of mathematical models and equations for expression of microbial growth in food and culture media have been developed. Many of the models are based on some basic mathematical models such as the logistic model and Gompertz model. Growth curves of organisms are often described well with the logistic model (Verhulst, 1838; Pearl, 1927; Vadasz et al., 2001). Also, it is quite natural that the growth kinetics of microbes is expressed with a differential equation, similar to other natural phenomena. The rate of growth, $\mathrm{d}N/\mathrm{d}t$, by the logistic model is written in a differential equation form as follows:

$$dN/dt = rN(1 - N/N_{\text{max}}), \tag{1}$$

where N is the population (arithmetic number) of the organism at time t. r is the rate constant, or the

maximum specific growth rate. $N_{\rm max}$ is the maximum population (at the stationary phase), which is often referred to as the carrying capacity of the environment. Here, $N_{\rm max}$ is an asymptote; N can be very close to, but cannot be that value. The logistic model contains the term, $1-N/N_{\rm max}$, which suppresses the rate of growth at a high population. When N is very small (during the lag phase), the value of this term is almost one, and thus does not affect the rate of growth. As N increases to approach $N_{\rm max}$, the value approaches zero, thus making the rate of growth almost zero (during the stationary phase). A growth curve described with this model is sigmoid on an ordinary Cartesian plane.

Bacterial growth curves are generally sigmoid on a semi-logarithmic plot. The logistic model, however, cannot generate a sigmoid curve on a semi-logarithmic plot. The model generates a convex curve consisting of a monotonously increasing portion and a stabilizing one, without a lag phase at the initial period (Vadasz et al., 2001). The logistic model, therefore, cannot be applied to bacterial growth. Some efforts have been made to overcome this shortcoming of the original logistic model (Hutchinson, 1948; Gibson et al., 1987). Hutchinson

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Nomenclature		c	adjustment factor	
		T	temperature	
N	cell population	SSE	sum of squared errors between the cell	
t	incubation time		populations predicted and those measured	
r	the rate constant of growth		(log unit) at the observation points	
$N_{\rm max}$	the maximum population (at the stationary	MSS	mean of SSE	
	phase)	n	number of observation points	
$N_{ m min}$	the minimum population	R	the correlation coefficient of linearity	
N_0	the inoculum size		·	
k	the rate constant of growth measured at the			
	exponential phase			

(1948) introduced a term of time delay in the logistic equation (1). Gibson et al. (1987) modified the logistic model to fit the bacterial growth data as follows:

$$\log N = A + C/\{1 + \exp(-B(t - M))\},\tag{2}$$

where A, C, B, M are parameters and exp is an exponential function. In Eq. (2), N and t in the original equation were transformed to $\log N$ and t-M, respectively, to fit a sigmoidal growth curve on a semilogarithmic plot. Similarly, Gibson et al. (1987) proposed a modified Gompertz model for bacterial growth. The modified logistic and Gompertz models fit bacterial growth, but the latter model gave better results (Gibson et al., 1987, 1998). The modified Gompertz model, thus, has been studied by a number of investigators and used in predictive microbiology software programs such as the Pathogen Modeling Programs (http://www.arserrc. gov/mfs/) and the Food Micromodel (McClure et al., 1994), which are internationally well known. These modified models are practical, but strictly speaking, they are mechanically or kinetically unacceptable. Baranyi and Roberts (1995) criticized that in a mechanical sense the modified Gompertz "function" should not be called as Gompertz model and not be used to fit the logarithm of the bacterial concentration in any form. The same criticism can be applied to the modified logistic model.

Baranyi et al. (1993) reported a new mathematical model for bacterial growth. This model is a combination of the logistic model and the Michaelis–Menten model. While the model is complex, it successfully fit bacterial growth (Baranyi et al., 1993). Buchanan et al. (1997) also proposed a three-phase linear model. This is a simplified model and successfully described bacterial growth.

One of the most important environmental factors that affect bacterial growth in food is the temperature. The temperature during storage and distribution of food products is constantly changing. An effective model that can well describe bacterial growth under dynamic temperature conditions are needed for practical use. Several investigators have developed mathematical models for dynamic temperatures, but the models' performances have not always been satisfactory

(Taoukis and Labuza, 1989; Fu et al., 1991; Baranyi et al., 1995; Brocklehurst et al., 1995; Van Impe et al., 1995; Alavi et al., 1999; Bovill et al., 2000, 2001; Koutsoumanis, 2001).

Under these circumstances we developed a new, simple mathematical model for bacterial growth, which was based on the logistic model, in this study. This new logistic model was then found to be useful for bacterial growth prediction at constant and dynamic temperatures.

2. Materials and methods

2.1. Micro-organism

Escherichia coli 1952 was isolated from a food source in our laboratory. The organism was identified following the FDA protocols (Anoymous, 1993).

2.2. Sample preparation

Bacterial cells were cultured on a freshly prepared nutrient agar plate (Nissui Pharmaceuticals, Tokyo, Japan) at 35°C for 24 h. Cells from several well-grown colonies were then transferred to a 5-ml portion of nutrient broth (Nissui Pharmaceuticals) with shaking (160 strokes/min) at 35°C for 24 h. Cultured cells were washed twice with 0.1 m phosphate–0.05 m citrate buffer, pH 7.0, with 0.005% Tween 80 by centrifugation at $6200 \times g$ and 10°C for 15 min. Cells were resuspended in a 5-ml portion of the buffer. This gave a cell suspension of approximately 10^{10} cfu/ml. The suspension was stored at about 4°C before use.

2.3. Incubation

The cell suspension prepared above was diluted to $1:10^6$ with the nutrient broth to achieve an initial level of 10^4 cfu/ml. For experiments on the effect of initial cell concentration, cell suspensions of 10^2 , 10^3 , 10^4 , and 10^5 cfu/ml were prepared in a similar manner. 3.5-ml portions of the cell suspension were transferred to

individual Pyrex screw-cap test tubes (10 mm I.D. × 100 mm) using a pipette (Fujikawa et al., 2000). Tightly capped tubes were then placed in a test tube rack. For the constant temperature experiment, the rack was placed in a water bath unit (DH-12, Taitec Corporation, Koshigaya, Japan) that was set at a given temperature ranging from 27.6°C to 36.0°C. The surface of the suspension in each test tube was 4 cm below that of the circulating water in the bath (Fujikawa et al., 2000). For a dynamic temperature experiment, the tubes in the rack were placed in a programmed incubator (PR-3G, Tabai Espec Corporation, Osaka, Japan). At each given interval of incubation at the constant and dynamic temperatures, two test tubes were taken and cooled in ice water.

The temperature histories of the cell suspensions in test tubes at constant and dynamic temperatures were monitored using a digital thermometer (AM-7002, Anritsu Meter, Tokyo) (Fujikawa et al., 2000). For a constant temperature experiment, the come-up time of the suspension temperature to a designated temperature was measured, which was between 5 and 30 s. Immediately after the come-up time at each temperature the initial time samples (t=0) were taken. For the dynamic temperatures, the temperature of the suspension was monitored at one-minute intervals. The come-up time was not taken into consideration in the dynamic temperature experiments, because the temperature history during the come-up time was part of the entire dynamic temperature history.

2.4. Cell counts

The cell counts in the cell suspensions (three per sample) were measured with the standard plate count agar method. Namely, the sample suspension was diluted with saline (0.85% sodium chloride solution) and incubated with the standard plate count agar (Eiken Chemicals, Tokyo) at 35°C for 48 h. The measured cell counts of the samples were transformed to the common logarithm with base 10. Averages and standard deviations of the transformed values were then estimated, to take the variability in bacterial cell counts into consideration.

2.5. Modeling

The new logistic model is based on the logistic model described with Eq. (1). During the initial period of incubation, bacterial cells need physiological adaptation to their new environment (Baranyi et al., 1993) and consequently their apparent rate of growth during this period is markedly lowered. To represent this, we assumed that the rate of growth of bacterial cells is also controlled by a factor related to the minimum cell concentration, N_{\min} Here N_{\min} is almost equal to the

initial cell concentration observed (the inoculum size), N_0 , of the sample. Namely, we assumed that the rate of growth would be also proportional to a term, $1-N_{\rm min}/N$, which was an "inverse" analogy of the term $1-N/N_{\rm max}$ in the original logistic model (1). The rate of growth of the new logistic model, therefore, is expressed as follows:

$$dN/dt = rN(1 - N/N_{\text{max}})(1 - N_{\text{min}}/N)^{c}.$$
 (3)

Here $c \geq 0$ is an adjustment factor and the rate constant, r is a function of temperature, T. In this model, N increases between the two asymptotes of N_{\min} and N_{max} with time. (But N cannot be N_{min} or N_{max} .) N_{\min} needs to be a bit smaller than N_0 to keep the value of the new term positive and avoid dN/dt < 0. When N is near N_{\min} during the lag phase, the value of this new term is very small, thus making the rate of growth very low. As N increases to approach N_{max} , the value approaches one during the stationary phase. On the other hand, the value of the term $1-N/N_{\text{max}}$ is almost one when N is very small (during the lag phase) and as N increases to approach N_{max} , the value approaches zero. Consequently, the rate of growth of the new model is strongly suppressed by the term $1-N_{\min}/N$ during the lag phase and by the term $1-N/N_{\text{max}}$ during the stationary phase.

2.6. Numerical solution of the model

Eq. (3) was solved numerically with the fourth-order Runge-Kutta method in the present study. The programming was performed using Microsoft Excel, a spread-sheet software.

Parameter r was set to be a measured rate constant of growth, k, at the exponential phase in an experimental curve. The exponential phase analysed is a straight portion, with a high linear correlation coefficient < 0.999 with Excel analysis, taken graphically from a curve on a semi-log plot. k is then calculated to be [the slope at the exponential phase] \times ln (10). In is a natural logarithm.

The values of $N_{\rm max}$ and N_0 were the observed values of an experimental curve. Each parameter value was transformed from the average of the log-transformed measured values to an arithmetic number for numeral calculation.

To start numerical calculation with the initial population measured (N_0) , N_{\min} needs to be slightly smaller than N_0 . This is because N_{\min} is an asymptote and N cannot be that value. In this study, thus, N_{\min} was estimated from the equation $N_{\min} = (1-1/10^6) \times N_0$. That is, N_{\min} was set to be 1 ppm smaller than N_0 . After calculation with a certain parameter values, a series of obtained N throughout an experiment were transformed to the common logarithm with base 10, to describe a growth curve on a semi-log plot. c was

determined as the value that minimizes the sum of the squared errors, SSE, between the predicted cell concentrations and those measured (log unit) at the observation points.

2.7. Comparison with other growth models

The value of k for a curve generated with the new logistic model was estimated from the slope at the exponential phase of the curve, as described above. (The curve itself is generated with r=k.) The lag period of the predicted curve, which is defined as a period between the initial point and a point where the regression line for the exponential phase intersects a horizontal line penetrating the initial point on the semilogarithmic plot (Dalgaard, 1995), was also estimated. Similarly, the values of k and lag period for experimental curves were estimated.

Experimental growth data were also analysed with the Baranyi and modified Gompertz models, by using a software program, DMFit. It was kindly obtained from Dr. J. Baranyi (http://www.ifr.bbsrc.ac.uk/Safety/DMFit/default. html). Here parameters mCurv and h_0 in the Baranyi model were set to be both 10, which is the default value. The Baranyi and modified Gompertz curves were generated with the parameter values obtained with the analysis. The values of k and lag period of the curves were estimated in the same way as that of the new model.

2.8. Statistical analysis

The mean of the squared errors between the predicted cell concentrations and those measured (log unit) at the observation points, MSS, was defined to be MSS = SSE/n as a measure of the goodness of fit. n is the number of observation points of an experiment.

3. Results

3.1. Bacterial growth at various initial concentrations

When *E. coli* cells at various initial concentrations ranging from 10² to 10⁵ cfu/ml were grown at 34.0°C, sigmoidal growth curves of cells were obtained. All growth curves were accurately described with the new logistic model (Fig. 1). The values of MSS for the curves were very small (Table 1).

The initial cell concentration of the bacterial suspension did not affect the values of parameters r, c, and $N_{\rm max}$ of the model (Table 1). The values of r for the growth curves were constant with an average of 2.1 ± 0.047 . In addition, the values of c for the curves were independent of the initial cell concentration and almost constant, with an average of 0.73 ± 0.028 . Values

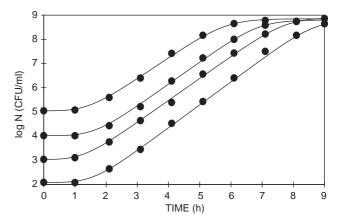


Fig. 1. Growth curves of E. coli 1952 at various initial cell concentrations ranging from 10^2 to 10^5 cfu/ml. Closed circles are the averages of the samples. The standard deviations for all samples were too small to show (as bars) in the graph. Lines describe the new logistic model.

Table 1
Parameter values of the new logistic model for the growth curves at various initial concentrations in Fig. 1

N_0 (cfu/ml)	10 ^{2.1}	10 ^{3.0}	10 ^{4.0}	10 ^{5.1}
r (1/h)	2.2	2.1	2.1	2.0
c	0.74	0.69	0.75	0.71
N _{max} (cfu/ml)	$10^{8.8}$	$10^{8.9}$	$10^{8.8}$	$10^{8.9}$
MSS (log unit)	0.0077	0.0048	0.0040	0.0032

of N_{max} were also constant, ranging from $10^{8.8}$ to $10^{8.9}$ cfu/ml.

3.2. Bacterial growth at various constant temperatures

When $E.\ coli$ cells were grown at constant temperatures from 27.6°C to 36.0°C, sigmoidal growth curves of the cells were also well described with the new logistic model. One of the examples was shown in Fig. 2. Parameter values of the model for the curves are shown in Table 2. The values of MSS for the curves were also very small (Table 2). The value of r of the model changed with the temperature, T(K), and the Arrhenius analysis for r was very linear with a correlation coefficient of linearity of 0.999 (Fig. 3). The linear regression line in the figure was described as follows:

$$\ln r = 21.0 - 6230/T.$$
(4)

The value of c was constant with an average of 0.72 ± 0.021 (Table 2). $N_{\rm max}$ was also constant at the chosen temperatures, ranging from $10^{8.8}$ to $10^{8.95}$ cfu/ml (Table 2).

3.3. Comparison with other growth models

The new model was compared for *E. coli* growth curves at various constant temperatures studied above

with other growth models of the Baranyi and modified Gompertz models. The rate constant (k) at the exponential phase and the lag period predicted by the models were compared as critical measures for the model comparison.

The three models well described the growth curves. One of the results was shown in Fig. 4. A curve predicted with one model crossed over the others several times during the growth period. When observed in detail, curves predicted with the new model were almost the same as those with the Baranyi model, especially at the exponential and the stationary phases. The Gompertz curves were more variable throughout the growth.

The new model and the Baranyi model estimated the rate constant (k) and the lag period of bacterial growth more accurately than the Gompertz model did (Fig. 5). Linear regression analysis between the predicted and observed values also showed the same results (Table 3). The Gompertz model overestimated the rate constant and lag period. Similar results were obtained for the growth curves at various initial concentrations in Fig. 1 (data not shown). These results showed that the new

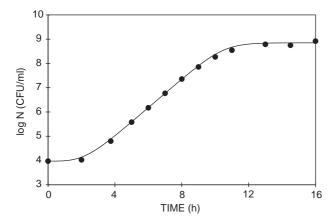


Fig. 2. Growth curve of *E. coli* 1952 at 27.6° C. The initial cell concentrations were all 10^{4} cfu/ml. Closed circles are the averages of the samples. The standard deviations of the samples are shown as bars. When the deviations for the samples were too small, the bars could not be drawn in the graph. Lines describe the new logistic model.

model predicted the *E. coli* growth curves successfully, similar to the Baranyi model and better than the modified Gompertz model.

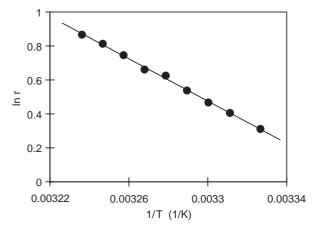


Fig. 3. The Arrhenius analysis for the rate constant of the model. Closed circles were obtained by analysing the growth curves at constant temperatures ranging from 27.6°C to 36.0°C. The straight line is the linear regression line.

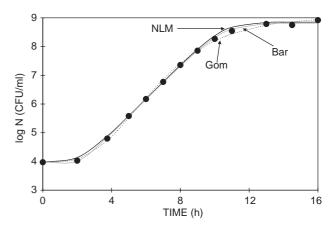


Fig. 4. Comparison of growth prediction by the new logistic model with those by the Baranyi and Gompertz models. *E. coli* growth curve at 27.6°C shown in Fig. 2 was analysed with the models. Closed circles are experimental. Abbreviations: NLM, the new logistic model (solid line); Bar, Baranyi model (gray line); Gom, Gompertz model (dotted line).

Table 2
Parameter values of the new logistic model for the growth curves at various constant temperatures in Fig. 2

	Temperature (°C)								
	27.6	29.0	30.0	31.0	32.0	33.0	34.0	35.0	36.0
r (1/h)	1.37	1.50	1.60	1.71	1.87	1.94	2.11	2.26	2.38
c	0.73	0.71	0.76	0.69	0.71	0.71	0.74	0.72	0.74
N_0 (cfu/ml)	$10^{4.0}$	$10^{3.9}$	$10^{4.0}$	$10^{3.9}$	$10^{3.9}$	$10^{3.9}$	$10^{4.0}$	$10^{3.9}$	$10^{3.7}$
$N_{\rm max}$ (cfu/ml)	$10^{8.85}$	$10^{8.85}$	$10^{8.85}$	$10^{8.8}$	$10^{8.9}$	$10^{8.9}$	$10^{8.8}$	$10^{8.95}$	$10^{8.9}$
MSS (log unit)	0.0051	0.0020	0.0028	0.0036	0.0071	0.0030	0.0048	0.0033	0.0045

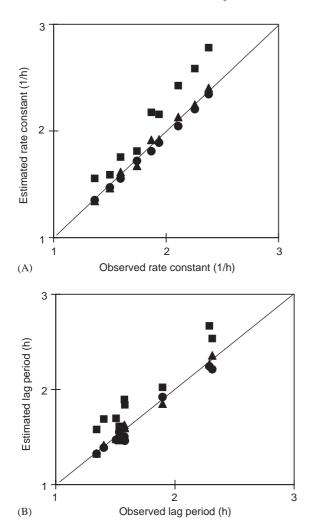


Fig. 5. Comparison of predictions of the rate constant (A) and the lag period (B) by the new logistic model with those by the Baranyi and Gompertz models. *E. coli* growth curves at $27.6-36.0^{\circ}$ C were analysed with the models. Symbols: \bullet , the new logistic model; \blacktriangle , the Baranyi model; \blacksquare , the Gompertz model. Straight lines are the lines of equivalence.

3.4. Bacterial growth at dynamic temperatures

 $E.\ coli$ growth at a dynamic temperature was predicted numerically using the new logistic model (Eq. (3)) with the recorded temperature history of a sample suspension. The temperature range of a dynamic temperature was within the range at the constant temperatures studied above. Prediction at a dynamic temperature was performed using the values of parameters $r,\ c,\ N_{\rm min},\$ and $N_{\rm max}$ studied at the constant temperatures. Namely, the value of r at the temperature of a time interval during an experimental temperature history was obtained from the Arrhenius model (Eq. (4)). For parameter c, the average at the constant temperatures (0.72) was used. $N_{\rm min}$ was determined from a measured inoculum size with the reduction ratio for each experiment. The value of $N_{\rm max}$ was fixed to

Table 3
Linear regression analyses for the rate constant and lag period predicted with the new logistic, Baranyi, and Gompertz models in Fig. 5

Model	Slope	Intercept	R^2
A. Rate constant			
The new logistic	0.972	0.0133	0.998
Baranyi	1.04	-0.0814	0.992
Gompertz	1.27	-0.272	0.978
B. Lag period			
The new logistic	0.962	0.0253	0.982
Baranyi	1.01	-0.0292	0.989
Gompertz	1.06	0.135	0.948

Data in Fig. 5 were analysed with Microsoft Excel.

10^{8.9} cfu/ml. Finally, a series of the measured temperature data during the experiment were embedded into the numerical solution program.

Various types of a dynamic temperature history were studied for bacterial growth prediction. Dynamic temperatures with various intervals were studied. For all dynamic temperature histories, the new logistic model successfully described the experimental growth of the micro-organism (Fig. 6). MSS values for the growth curves in Figs. 6A–C were small enough, being 0.0096, 0.011, and 0.0039 (log unit), in order. These results showed that the model had a potential to describe bacterial growth curves at various types of a dynamic temperature.

3.5. Modeling of other bacterial growth

We studied the usefulness of the new model for other bacterial growth data reported so far such as Gibson et al. (1988). One of the examples was shown in Fig. 7. The model successfully described the bacterial growth curves. When the model was compared with the Baranyi and modified Gompertz models, the same results as those observed for *E. coli* were found (Fig. 7 and Table 4).

4. Discussion

The real mechanism of the physiological adaptation of bacterial cells to a new environment during the lag period is too complex to express with mathematical models at present. Therefore, a mathematical substitution for this period is needed in a growth model. A simpler substitution is better for numerical calculation and practical use. In the new model, the term $1-N_{\rm min}/N$, was introduced for the mathematical substitution. In this sense, the model would not be a mechanistic one. On the other hand, $N_{\rm max}$ in the original and new logistic

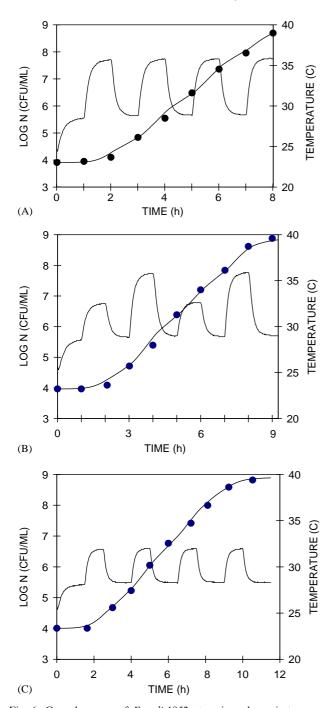


Fig. 6. Growth curves of E. coli 1952 at various dynamic temperatures. The initial cell concentrations were all $10^4\,\mathrm{cfu/ml}$. Closed circles are the averages of the samples. The standard deviations for the samples are shown as bars. When the deviations were too small, the bars could not be drawn in the graph. Thick lines describe the new logistic model. Thin lines are the temperatures of the sample suspensions.

models was introduced for the growth suppression during the stationary phase. The reason for the occurrence of this phase is not fully understood, but is suggested to be due to the lack of nutrients and/or the accumulation of harmful wastes from cells.

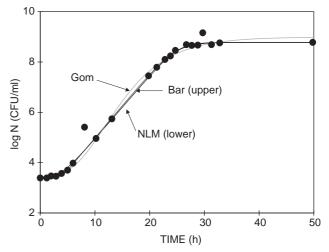


Fig. 7. Application of the three models to *Salmonellae* growth in Trpytone soya broth at 20°C from the data from Baranyi et al. (1993), which was originally from the data of Gibson et al. (1988). Closed circles show the experimental data. Abbreviations: NLM, the new logistic model (thick line); Bar, Baranyi model (thin line); Gom, Gompertz model (gray line).

Table 4
Rate constants and lag periods estimated by the new logistic model, the Baranyi model, and the modified Gompertz model for the growth curves in Fig. 7

Model	Rate constant (1/h)	Lag period (h)		
The new logistic	0.57	3.6		
Baranyi	0.58	3.9		
Gompertz	0.73	5.5		
Observed	0.58	3.7		

Values are estimated from growth curves generated with the models.

The rate constant of growth (k) and the lag period of predicted curves were chosen as measures for model comparison in this study. These measures which characterize the shape of a curve are thought to be critical for model comparison rather than curve fitting measures such as SSE (Baranyi and Roberts, 1995). Moreover, food producers have great practical interests in when the exponential phase of contaminants in their products begins (or how long the lag period is) and how fast they can grow at the exponential phase in their products.

The procedures of curve fitting for the Baranyi and the modified Gompertz models are different from those for the new logistic model. There are several parameters to be optimized in the Baranyi and Gompertz models. With experimental data, DMFit gives the optimal values of the rate constant of growth, lag period, and the initial and maximum cell concentrations of Baranyi and Gompertz curves. In the new logistic model, parameter c is the only adjusting factor for curve fitting and other parameters of r, N_{\min} , and N_{\max} are obtained from

experimental data, as described in Materials and methods. For precise analysis on a common base, therefore, the rate constant and the lag period for comparison were all estimated from curves generated by each model with its parameter values, in this study. That is, the values for the rate constant and the lag period directly obtained with the DMFit, which are very close to values estimated from the generated curves, were not used for comparison. As a result, the rate constant and the lag period predicted with the new logistic and the Baranyi models were closer to the experimental ones than those with the Gompertz model.

The modified Gompertz model overestimated the rate constant and the lag period in this study (Fig. 5 and Table 4). Overestimation of the rate constant by the model has been observed by many investigators (Whiting and Cygnarowicz-Provost, 1992; Membre et al., 1999). The model also overestimated the maximum cell population in comparison with the other two models and the experimental values, as shown in Figs. 4, 5, and 7. The results in Figs. 4 and 7 that the Gompertz curves are more variable than the Baranyi curves are also reported by Baranyi (1997). The Baranyi model successfully predicted *E. coli* and *Salmonellae* growths in this study. When observed in detail, this model gives a more straight increase at the beginning of the exponential phase than the other two models (Fig. 4).

The new model is essentially different from the modified logistic model (Gibson et al., 1987), but the two models originated from the logistic model, as described in the Introduction. In a preliminary study, thus, the new model was compared with the modified model in description of bacterial growth. The methods of analysis were similar to those of the modified Gompertz model. The modified logistic model well described growth curves of E. coli studied here, but the curves were more variable over the growth period than those generated with the new logistic model. One of the examples was shown in Fig. 8. Durations of the lag, exponential, and stationary phases of the curve were not graphically clear (Fig. 8). This feature of the modified logistic model was similar to that by the modified Gompertz model studied above. Therefore, it was concluded that the new model might be superior to the modified one in description of growth curve.

Parameter c, which is introduced as an adjustment factor, shifts a sigmoidal curve parallel to the time axis; as the value of the parameter is smaller, the curve shifts to the more left side, and vice versa. That is, with a smaller value of c, the model describes a growth curve with a shorter lag period, by making the effect of the term $1-N_{\rm min}/N$ smaller in the equation. When c=0, the new model (Eq. (3)) is mathematically equal to the original logistic model (Eq. (1)) which produces a growth curve without a lag phase on a semi-logarithmic plot, as described in the Introduction section.

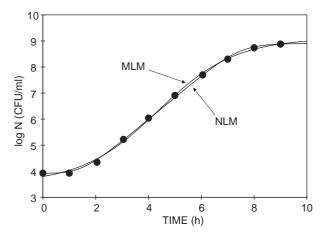


Fig. 8. Comparison of growth prediction between the new and modified logistic models. *E. coli* growth at 33°C was analysed with the models. Closed circles are experimental. Abbreviations: NLM, the new logistic model (solid line); MLM, the modified logistic model by Gibson et al. (gray line).

The value of c was affected by the difference between the values of parameter N_{\min} and the measured initial concentration, N_0 , of a sample. When the difference between N_{\min} and N_0 is great, a correlation between cand was N_{\min} observed. That is, when N_{\min} was set to make the difference not small enough, such as $1/10^3$, the value of c changed with the initial cell concentration and the constant temperature studied (data not shown). As the difference between N_{\min} and N_0 was smaller, the value of c was more stable. In this study, thus, the difference was set to be much smaller, which was $1/10^6$. Using this value for the difference, a stable value of c (0.72+0.021) was obtained at constant temperatures (Table 2). Using this value of c, the new model successfully predicted E. coli growth for various dynamic temperatures, as shown in Fig. 6.

The new logistic model successfully described growth curves of bacteria under various conditions other than the results shown in the present study (results not shown). For example, it predicted well *Salmonellae* growth under various conditions of constant temperature and pH reported by Gibson et al. (1988), the data being provided by Baranyi et al. (http://www.ifr.bbsrc.ac.uk/ Safety/DMFit/default.html). In our preliminary study, the model successfully described *Staphylococcus aureus* growth in milk as well.

The new model successfully predicted *E. coli* and *Salmonellae* growth curves for various patterns of the temperature history in this study. This suggests that the model could be a useful tool for bacterial growth prediction for various temperature histories. When a software program consisting of the model is embedded in a temperature-recording device, such a device could predict bacterial growth in a food product from the temperature history of the product during the storage and transportation processes.

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References

- Alavi, S.H., Puri, V.M., Knabel, S.J., Mohtar, R.H., Whiting, R.C., 1999. Development and validation of a dynamic growth model for Listeria monocytogenes in fluid whole milk. J. Food Prot. 62, 170–176.
- Anonymous, 1993. Standard Methods of Analysis in Food Safety Regulation. Japan Food Hygiene Association, Tokyo, pp. 79–91.
- Baranyi, J., 1997. Simple is good as long as it is enough. Food Microbiol. 14, 189–192.
- Baranyi, J., Roberts, T.A., 1995. Mathematics of predictive food microbiology. Int. J. Food Microbiol. 26, 199–218.
- Baranyi, J., Roberts, T.A., McClure, P., 1993. A non-autonomous differential equation to model bacterial growth. Food Microbiol. 10, 43–59.
- Baranyi, J., Robinson, T.P., Kaloti, A., Mackey, B.M., 1995. Predicting growth of *Brochothrix thermosphacta* at changing temperature. Int. J. Food Microbiol. 27, 61–75.
- Bovill, R., Bew, J., Cook, N., D'Agostino, M., Wilkinson, N., Baranyi, J., 2000. Predictions of growth for *Listeria monocytogenes* and *Salmonella* during fluctuating temperature. Int. J. Food Microbiol. 50, 157, 165.
- Bovill, A.R., Bew, J., Baranyi, J., 2001. Measurement and predictions of growth for *Listeria monocytogenes* and *Salmonella* during fluctuating temperature. II. Rapidly changing temperatures. Int. J. Food Microbiol. 67, 131–137.
- Brocklehurst, T.F., Mitchell, G.A., Ridge, Y.P., Seale, R., Smith, A.C., 1995. The effect of transient temperatures on the growth of *Salmonella typhimurium* LT2 in gelatin gel. Int. J. Food Microbiol. 27, 45–60
- Buchanan, R.L., Whiting, R.C., Damert, W.C., 1997. When is simple good enough: a comparison of the Gompertz, Baranyi, and threephase linear models for fitting bacterial growth curves. Food Microbiol. 14, 313–326.
- Dalgaard, P., 1995. Modelling of microbial activity and prediction of shelf life for packed fresh fish. Int. J. Food Microbiol. 26, 305–317.

- Fu, B., Taoukis, P.S., Labuza, T.P., 1991. Predictive microbiology for monitoring spoilage of dairy products with time-temperature integrators. J. Food Sci. 56, 1209–1215.
- Fujikawa, H., Morozumi, S., Smerage, G.H., Teixeira, A.A., 2000. Comparison of capillary and test tube procedures for analysis of thermal inactivation kinetics of mold spores. J. Food Prot. 63, 1404–1409.
- Gibson, A.M., Bratchell, N., Roberts, T.A., 1987. The effect of sodium chloride and temperature on the rate and extent of growth of *Clostridium botulinum* type A in pasteurized pork slurry. J. Appl. Bacteriol. 62, 479–490.
- Gibson, A.M., Bratchell, N., Roberts, T.A., 1988. Predicting microbial growth: growth responses of *Salmonella* in a laboratory medium as affected by pH, sodium chloride and storage temperature. Int. J. Food Microbiol. 6, 155–178.
- Hutchinson, G.E., 1948. Circular casual systems in ecology. Ann. N. Y. Acad. Sci. 50, 211–246.
- Koutsoumanis, K., 2001. Predictive modeling of the shelf life of fish under nonisothermal conditions. Appl. Environ. Microbiol. 67, 1821–1829.
- McClure, P.J., Blackburn, C.W., Cole, M.B., Curtis, P.S., Jones, J.E., Legan, J.D., Ogden, I.D., Peck, M.W., Roberts, T.A., Sutherland, J.P., Walker, S.J., 1994. Modelling the growth, survival and death of microorganisms in foods: the UK Food Micromodel Approach. Int. J. Food Microbiol. 23, 265–275.
- Membre, J.-M., Ross, T., McMeekin, T., 1999. Behavior of *Listeria monocytogenes* under combined chilling processes. Lett. Appl. Microbiol. 28, 216–220.
- Pearl, R., 1927. The growth of populations. Q. Rev. Biol. II 4, 532–548.
- Taoukis, P.S., Labuza, T.P., 1989. Applicability of time-temperature indicators as shelf life monitors of food products. J. Food Sci. 54, 783-788
- Vadasz, A.S., Vadasz, P., Abashar, M.E., Gupthar, A.S., 2001.
 Recovery of an oscillatory mode of batch yeast growth in water for a pure culture. Int. J. Food Microbiol. 71, 219–234.
- Van Împe, J.F., Nicola, B.M., Schellekens, M., Martens, T., De Baerdemaeker, J., 1995. Predictive microbiology in a dynamic environment: a system theory approach. Int. J. Food Microbiol. 25, 227–249.
- Verhulst, P.F., 1838. Notice sur la loi que la population suit dans son accroissement. Corr. Math. et Phys. Publ. par A. Quetelet. T X, 113–121.
- Whiting, R.C., Cygnarowicz-Provost, M., 1992. A quantitative model for bacterial growth and decline. Food Microbiol. 9, 269–277.