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A heterogeneous population model for the analysis of bacterial growth kinetics

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Abstract

A two-compartment, heterogeneous population model (HPM) was derived using the simulation software SB ModelMaker© to describe the growth of *Listeria monocytogenes* in bacteriological media at 5–35°C. The model assumed that, at time $t = 0$, the inoculum was distributed between two distinct compartments, Non-Growing and Growing, and that growth could be described by four parameters: initial total cell population (N_0), final maximum cell population (N_{max}), maximum specific growth rate (μ_{max}), and initial cell population in the Growing compartment (G_0). The model was fitted to the data by optimizing the four parameters, and lag phase duration (λ) was calculated. The resulting values of μ_{max} and λ were similar to those determined using the modified Gompertz equation. A new parameter, w_0, was defined which relates to the proportion of the initial cell population capable of growth, and is a measure of the initial physiological state of the cells. A modified model in which μ_{max} was replaced with a temperature function, and w_0 replaced G_0, was used to predict the effect of temperature on the growth of *L. monocytogenes*. The results of this study raise questions concerning the current definition of the lag phase. © 1997 Elsevier Science B.V.

Keywords: Predictive microbiology; Heterogeneous; Model; *Listeria monocytogenes*; Growth; Simulation

1. Introduction

Predictive microbiology, the use of mathematical models to describe the growth and death kinetics of foodborne microorganisms, has been an area of considerable activity over the last decade. Recent reviews have highlighted some of the advantages and disadvantages of this approach (McMeekin et al., 1993; Ross and McMeekin, 1994; Skinner et al., 1994). In their summary, Ross and McMeekin (1994) suggest that predictive models derived from model systems can be successfully applied to food systems, and that predictive microbiology can provide a rational framework for understanding the microbial ecology of food. Predictive models can be used to provide information for quantitative risk assessment, and thus may play a crucial role in the

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development of Hazard Analysis Critical Control Point (HACCP) systems (Baker, 1995; Notermans et al., 1995).

The usual approach to modelling bacterial growth involves fitting of bacterial count data to sigmoidal equations such as the modified Gompertz to obtain values for the Gompertz parameters B and M, which are used subsequently to calculate lag time (λ) and maximum specific growth rate (μ_{max}) (Gibson et al., 1988; Buchanan and Phillips, 1990). Models are then derived relating growth parameters to environmental factors such as temperature, pH and a_w using a variety of techniques which include Arrhenius equations (Davey, 1993), square root models (Ross, 1993), and response surface models (Gibson et al., 1988; Buchanan and Phillips, 1990).

One of the difficulties experienced with the use of empirical models for bacterial growth such as the modified Gompertz is the poor estimates often obtained for the lag time (Ratkowsky et al., 1991; Duh and Schaffner, 1993; Grau and Vanderlinde, 1993; Houtsma et al., 1994). This situation is exacerbated by the observations that lag time is affected by temperature history (Buchanan and Klawitter, 1991; Hudson, 1993) and by temperature fluctuations (Li and Torres, 1993; Zwietering et al., 1994b). Some workers have attempted to alleviate this problem by using the second derivative of the Gompertz equation to more accurately determine the lag time (Buchanan and Cygnarowicz, 1990). Others have concentrated their efforts towards deriving more mechanistic models for bacterial growth. Hills and coworkers (Hills and Wright, 1994; Hills and Mackey, 1995) have recently described the development of a structured-cell kinetic model, while Baranyi and coworkers (Baranyi et al., 1993a,b; Baranyi and Roberts, 1994, 1995; Baranyi et al., 1995) have taken a dynamic approach to model the physiological state of the cell. Definitions of the lag time have been reviewed recently by Zwietering et al. (1994b).

In the present study, a dynamic modelling approach was taken. The initial cell population was assumed to consist of at least two distinct populations, which were modelled independently. The resulting model provides a good fit to *Listeria monocytogenes* growth data at a variety of temperatures, and suggests that a novel approach to modelling bacterial growth may shed further light on the physiological state of the cells during the lag phase.

2. Materials and methods

2.1. Strains and culture conditions

L. monocytogenes Scott A was obtained from Dr. J. M. Farber, Health Protection Branch, Health Canada. The strain was subcultured twice in Tryptic Soy Yeast Extract Broth [TSBYE; Tryptic Soy Broth (Difco Laboratories, Detroit, MI) supplemented with 0.6% (w/v) Yeast Extract (Difco)] for 24 h at 30°C. Stock cultures were prepared in TSBYE plus 15% w/v glycerol, and were frozen in 0.2 ml volumes in cryovials at −25°C.

The contents of one cryovial of *L. monocytogenes* was transferred to 10 ml of TSBYE in a 50 ml Erlenmeyer flask. The flask was incubated for 24 h at 30°C with shaking at 150 rpm in a G86 Aquatherm Water Bath Shaker (New Brunswick Scientific, Edison, NJ). The culture was transferred (1%) to 10 ml fresh TSBYE and incubated under the same conditions for exactly 16 h. The resulting culture was used as the inoculum for growth experiments.

Growth experiments were carried out at 5, 10, 15, 20, 25, 30, and 35°C in 50 ml TSBYE in a 150 ml serum flask with shaking at 150 rpm. The medium was equilibrated to the appropriate temperature in a G86 Aquatherm Water Bath Shaker or G76D Gyrotory Water Bath Shaker (New Brunswick Scientific, Edison, NJ), with cooling supplied by the RF10 Frigidflow Bath Circulator (New Brunswick Scientific) as required. Cultures were inoculated to give an initial cell density of $\approx 10^4$ cfu · ml^{-1}. Cells were enumerated at appropriate intervals by diluting in 0.1% Bacto Peptone (Difco) and spread plating on Tryptic Soy Yeast Extract Agar [TSAYE; TSBYE plus 1.5% w/v Bacto Agar (Difco)] to determine the total cell count. Plates were incubated 72 h at 30°C.

2.2. Development of model

The heterogeneous population model (HPM) was developed using SB ModelMaker© Version 2.0 (Cherwell Scientific Publishing, Oxford, UK). A two-compartment model was proposed, with the assumption that the cell population at $t = 0$ (t_0) was

defined as N_0, and was distributed between Non-growing (NG) and Growing (G) compartments. It was further assumed that the concentration of NG cells did not change over the course of the experiment. Thus:

$$\frac{dNG}{dt} = 0; NG(t) = NG(0) = NG_0 \quad (1)$$

It was further assumed that the G cells were capable of exponential growth starting at t_0, thus representing that proportion of N_0 which was fully adapted to the new growth conditions. A logistic function was used to limit the total cell population to a maximum (N_{max}) defined by the stationary phase. Thus:

$$\frac{dG}{dt} = G \cdot \mu_{max}\left(1 - \frac{G}{N_{max}}\right); G(0) = G_0 \quad (2)$$

where μ_{max} = maximum specific growth rate.

The initial number of cells in the non-growing compartment was defined as:

$$NG_0 = N_0 - G_0 \quad (3)$$

Since the differential equations describe the behaviour of real cell populations rather than the logarithms, two variables were defined in order to visualize the data after the simulation. The logarithm of the total cell population at time = t was the logarithm of the sum of the number of cells in the two compartments:

$$\log N(t) = \log(NG(t) + G(t)) \quad (4)$$

The logarithm of the growing cells at time = t was the logarithm of the number of cells in the G compartment:

$$\log G(t) = \log(G(t)) \quad (5)$$

Optimized values for the four parameters N_0, N_{max}, μ_{max} and G_0 were obtained by least squares fitting. There was some difficulty in fitting the HPM to experimental data using SB ModelMaker, thus fitting was carried out using Scientist® (Micromath Scientific Software, Salt Lake City, UT, USA). A modified Powell algorithm was used to minimize the sum of squared deviation between observed data and model calculations. Initial parameter estimates were obtained using simplex optimization. Differential equations were solved numerically by the method of Runge–Kutta, since the software does not require analytical forms of equations. Subsequent simulations with derived models were carried out using SB ModelMaker.

Growth data were also fitted to the modified Gompertz equation described by Willocx et al. (1993):

$$\log N(t) = A + C \exp[-\exp((2.718 \cdot (R_g/C) \cdot (\lambda - t) + 1)] \quad (6)$$

where $A = \log N_0$ ($\log \text{cfu} \cdot \text{ml}^{-1}$); C = asymptotic increase in population density ($\log \text{cfu} \cdot \text{ml}^{-1}$); R_g = growth rate (h^{-1}); λ = lag phase duration (h).

Note that since data are plotted on a $\log_{10}$ rather than a natural log scale, R_g and μ_{max} are related by:

$$R_g = \frac{\mu_{max}}{\ln 10} \quad (7)$$

3. Results

The HPM was fitted to each of the data sets using Scientist, and the results are presented in Table 1. Excellent fits were obtained for all temperatures. An example of the fit obtained with the 5°C data is shown in Fig. 1. The solid line represents the model fit for log N, while the broken line represents the simulated values for log G. Clearly, representing log N as the sum of the NG and G compartments results in a smooth transition from the lag to the exponential phase.

Results from the HPM were compared with those derived from fits using the Gompertz equation. R_g values were obtained directly from the Gompertz, or, in the case of the HPM, were derived from μ_{max} using Eq. (7). The Gompertz model gave values for λ directly. Values for λ for the HPM were derived from the following equation:

$$\log N_0 = \log G_0 + \lambda R_g \quad (8)$$

$$\lambda = \frac{\log N_0 - \log G_0}{R_g} \quad (9)$$

The square root model was used to describe the influence of temperature on μ_{max} and λ, and the results are shown in Fig. 2. Values for μ_{max} were slightly higher with the HPM, and values for λ were generally shorter than found with the Gompertz equation (Table 2). R-squared values for μ_{max}

Table 1
Results of fitting the heterogeneous population model (HPM) to experimental data for *Listeria monocytogenes*

Temp (°C)	N_0	N_{max}	G_0	μ_{max}	SSD	r^2
5	47 714	3.7×10^9	4883	0.049	0.261	0.999
10	23 849	8.4×10^9	6397	0.104	0.688	0.999
15	59 275	5.4×10^9	13 109	0.236	0.294	0.999
20	37 535	7.0×10^9	3224	0.443	0.085	0.999
25	51 102	7.6×10^9	10 692	0.652	0.163	0.999
30	52 929	4.4×10^9	9988	0.833	0.092	0.999
35	35 975	4.7×10^9	8181	1.170	0.070	0.999

N_0, initial cell population (cfu·ml^{-1}).
N_{max}, maximum cell population (cfu·ml^{-1}).
G_0, initial cell population in the Growing compartment (cfu·ml^{-1}).
μ_{max}, maximum specific growth rate (h^{-1}).
SSD, sum of squared deviations.

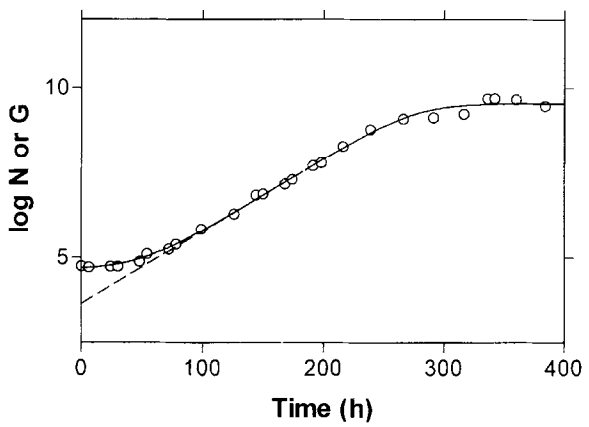

Fig. 1. Plot of the fitted variables Log N and Log G for experimental data at 5°C.

(0.9969 and 0.9962) and λ (0.9807 and 0.9740) were obtained for the HPM and Gompertz models, respectively. An equation describing the effect of temperature on μ_{max} was derived for the HPM:

$$\sqrt{\mu_{max}} = 0.08312 + 0.04487 \cdot T \qquad (10)$$

While calculating λ for the HPM using Eq. (9), it was noted that log N_0 − log G_0 appeared to be constant and independent of temperature. This value (herein defined as w_0) was calculated for each data set (Table 2). Several workers have observed that $\lambda \cdot \mu_{max}$ is constant under isothermal conditions (Baranyi and Roberts, 1994; Houtsma et al., 1994; Zwietering et al., 1994a), therefore this calculation was made for R_g and λ from the Gompertz fits (Table 2). Values for $\lambda \cdot R_g$ always exceeded w_0, and a paired t-test found this difference to be significant

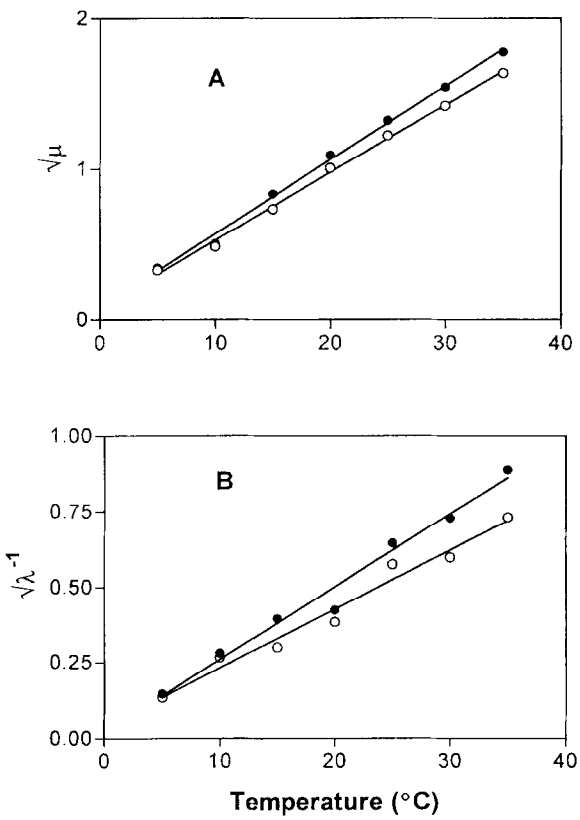

Fig. 2. Influence of temperature on the square root of (A) maximum specific growth rate (μ_{max}) and (B) reciprocal lag time (λ^{-1}) as determined by the modified Gompertz equation (○) and the heterogeneous population model (·).

($P \leq 0.05$). Baranyi and Roberts (1995) have defined the product of μ_{max} and λ as h_0. Thus, it is proposed that w_0 is related to h_0 by the following equation:

Table 2
Comparison of growth rate (R_g) and lag phase duration (λ) calculated using the HPM and the Gompertz equation

Temp (°C)	HPM			Gompertz		
	λ	R_g	w_0	λ	R_g	$\lambda \cdot R_g$
5	46.9	0.021	0.990	54.7	0.023	1.27
10	12.7	0.045	0.571	14.2	0.049	0.70
15	6.39	0.102	0.655	11.1	0.133	1.47
20	5.54	0.192	1.066	6.77	0.226	1.53
25	2.40	0.283	0.679	3.02	0.332	1.00
30	1.89	0.383	0.724	2.79	0.452	1.26
35	1.17	0.508	0.643	1.87	0.600	1.19
Mean			0.762			1.20
(s.e.)			(0.072)			(0.107)

s.e., standard error.

$$w_0 = \frac{h_0}{\ln 10} \tag{11}$$

In a manner analogous to that described by Baranyi et al. (1995) for *Brochothrix thermosphacta*, a revised model for *L. monocytogenes* was derived (Fig. 3). In this model, μ_{max} was replaced by the temperature function in Eq. (10), and G_0 was replaced by w_0. N_0 and N_{max} were entered by the user. During the simulation, G_0 was calculated from w_0 by:

$$w_0 = \log N_0 - \log G_0 \tag{12}$$

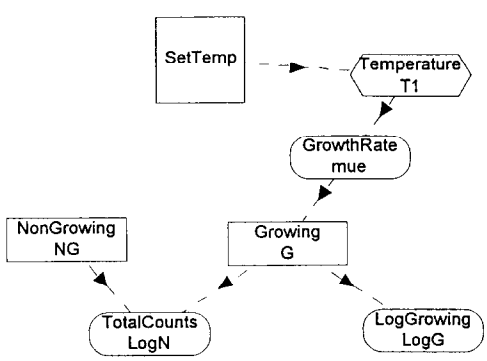

Fig. 3. Flow chart for modified two-compartment, heterogeneous population model for *Listeria monocytogenes*. The two compartments (represented by rectangular boxes) represent the numbers of Non-Growing and Growing cells at time = t. The variables (represented by rounded boxes) are used to calculate the logarithm of the Growing cells (Log G) and the logarithm of the total cell population (Log N). Maximum specific growth rate (μ_{max}) was replaced by a variable (Growth Rate) influenced by the temperature, which appears as a defined value set by the user.

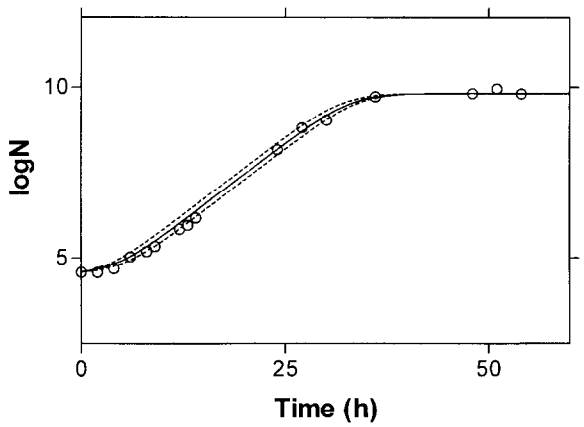

Fig. 4. Output of a simulation performed with the model in Fig. 3 with temperature set at 20°C. The simulated increase in log N (solid line) is compared to experimental data. The 95% confidence intervals of the model are represented by the broken lines.

with the value of w_0 taken from Table 2. NG_0 was calculated using Eq. (3). The model was also modified to allow the user to enter an appropriate temperature at the start of the simulation.

Fig. 4 shows the results of a simulation performed using the revised model at 20°C. The data points are the 20°C experimental values used to derive the model, and are included in this figure for illustrative purposes. The solid line represents the simulation output for log N, while the dotted lines represent the 95% confidence intervals calculated from the standard error for w_0 (Table 2).

4. Discussion

A simple, two-compartment heterogeneous population model (HPM) has been presented which effectively describes the growth kinetics of *L. monocytogenes* at 5–35°C. The model assumes that a growing bacterial population is dominated by the first cells to begin growth, a theory implicitly believed by many microbiologists, although as yet unproven (Whiting and Cygnarowicz-Provost, 1992). The model accounts for the gradual transition from lag to exponential growth, and normal variation in the model's w_0 parameter may explain the variations in apparent lag phase observed by many workers.

The HPM assumes that the concentration of NG cells is constant over the entire experimental period,

thus precluding any adaptation. This may not necessarily be the case under all conditions. The model in Fig. 3 could be expanded to include a flow of cells from NG to G, analogous to the first order adaptation process proposed by Whiting and Cygnarowicz-Provost (1992)however, preliminary attempts to include this characteristic in the model did not improve the fit (data not shown). The HPM predicts that any cells in the G compartment quickly dominate, whether they existed there at $t=0$ or arose by adaptation. The model further predicts that the contribution of cells adapting subsequently is minimal. Adaptation might play a greater role under conditions where G_0 is expected to be small, i.e., in a stressed population. It should be made clear that the cells referred to here as NG are only non-growing over the initial part of the experiment, that is, during the lag phase. All cells present at $t=0$, whether G or NG would, given sufficient time, form colonies on solid media.

We have no direct evidence to support the hypothesis that a fraction of the cells are in fact capable of exponential growth at $t=0$ ($G_0>0$); this is an assumption made to simplify the model fitting process. Thus, the HPM should be considered as an empirical model at present. Under some conditions, however, a proportion of the cell population may be capable of growth immediately after inoculation, for example when the new growth environment does not differ substantially from that under which the inoculum was grown.

Of the current models attempting to define the lag phase in physiological terms, the model proposed by Baranyi and coworkers (Baranyi et al., 1993a,b; Baranyi and Roberts, 1994, 1995; Baranyi et al., 1995) is the best documented. The present model shares some characteristics with the Baranyi model (Baranyi et al., 1993b). During the exponential phase both models give a straight line. In addition, the HPM, like the Baranyi model, does not require data from the stationary phase to achieve a satisfactory fit, in contrast with the Gompertz model. However, the Baranyi model uses pure exponential growth, while a logistic growth function was employed for the HPM.

The major difference between the two models is the assumption by the Baranyi model that the cell population is homogeneous (Baranyi et al., 1993b). In spite of this fundamental difference, the most intriguing similarity between these two models is that they both predict h_0, the initial physiological state of the culture. In the Baranyi model, the initial physiological state is embodied in a parameter α_0 which is related to h_0 (Baranyi and Roberts, 1994; Baranyi et al., 1995):

$$h_0 = -\ln \alpha_0 \tag{13}$$

This parameter, termed the suitability for growth, is a measure of the intracellular concentration of a critical metabolite, and is used to adjust the μ from 0 to μ_{max} (Baranyi et al., 1995). This has the effect of accounting for the gradual change in the cell population from the lag to the exponential growth phase. In the HPM, the parameter G_0 is analogous to α_0, and also describes the suitability for growth; however, in this case, the initial physiological state of the cells is embodied in only a sub-population of growing cells. The apparent gradual increase in log N during the transition from lag to exponential phase is a consequence of summing the NG and G cell compartments.

While the HPM in its present form is simple and provides good fits to experimental data, it appears to have some limitations. Difficulty was experienced when trying to fit some of the data sets using the ModelMaker program; convergence was not always obtained. The software program Scientist gave rapid convergence with all data sets. There was also some deviation between calculations of w_0 from the HPM, and the $\lambda \cdot R_g$ from the Gompertz. While significantly different, these two values were not markedly different. Other workers have shown that there is wide variation in measurement of λ (Ratkowsky et al., 1991; Duh and Schaffner, 1993; Grau and Vanderlinde, 1993; Houtsma et al., 1994). In addition, Baranyi et al. (1995) reported large standard errors in calculation of h_0 for *B. thermosphacta*. Thus, the differences noted with the HPM may not be important.

The present study also calls into question the concept of lag phase. The lag phase is normally defined as the intersection of the tangent to μ_{max} extrapolated to the lower asymptotic value (log N_0). Thus the HPM defines the number of cells present at λ (N_λ) as:

$$N_\lambda = NG_\lambda + G_\lambda \tag{14}$$

from Eq. (3), and from Eq. (4):

$$N_\lambda = (N_0 - G_0) + N_0 = 2N_0 - G_0 \tag{15}$$

Thus, the cell population is always $>N_0$ at the end of the lag phase as defined by conventional growth models and the HPM. Under certain environmental conditions, a foodborne pathogen present in a product might be considered to be in the lag phase, based on predictions made by existing models, and thus the product would be considered as safe. The above argument suggests, however, that an increase in cell numbers may have already taken place. Clearly, a new definition of the lag phase is required which is based on a measurable physiological event.

The HPM suggests that, at any time $t>0$, a proportion of the cell population is in exponential growth, and a constant threat might be implied. It must be emphasized, however, that there is no evidence for the existence of a growing sub-population at t_0. When one is concerned about a virulent foodborne pathogen, a small population of growing cells masked by a background of non-growing cells could present a considerable health hazard if they were capable, for example, of producing a toxin. Alternatively, exponential phase cells might be less resistant to stress factors such as stomach acid. Any assessment of the potential risk of a small growing population must include factors such as infectious dose and susceptibility of the pathogen in question to environmental stress.

Development of models in predictive food microbiology normally involves the derivation of an equation which defines the dependent variable (e.g., growth) as an explicit function of the independent variable, time. The resulting equations are fitted to the data using non-linear regression programs. In the present study, a simulation modelling approach was employed in which numerical solutions were derived for differential equations. Using software such as SB ModelMaker, it is possible to more clearly understand the interactions between the various compartments and variables of the model, and subsequently more complex models can be designed.

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