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Stochastic modelling of the growth of a microbial population under changing temperature regimes

T.K. Soboleva[a,*], A.E. Filippov[b], A.B. Pleasants[a], R.J. Jones[a], G.A. Dykes[a]

[a] *AgResearch, Ruakura, Private Bag 3123, Hamilton, New Zealand*
[b] *Donetsk Institute for Physics and Engineering of NASU, 340114 Donetsk, Ukraine*

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Abstract

The application of models of microbial growth to the design of food safety systems requires consideration of the effect of arbitrary changes in external variables on growth of bacteria. In particular, the effect of changes in external variables, such as temperature, on the probability that the microbial population size will not exceed acceptable levels at a given time needs to be predicted. This paper presents a method of calculating the time-dependent probability distribution of the microbial population size under arbitrary changes of temperature through time. To illustrate this method, the effect of a sudden temporary increase in temperature on the evolution of the probability distribution of *Lactobacillus plantarum* population size is presented. The effect of this change in temperature on the time taken for the population to reach a critical size, with a given probability, is also calculated and the application of this calculation to the design of HACCP protocols is discussed.

Keywords: Modelling; Microbial population; Probability distributions; Temperature shift

1. Introduction

Predictive microbiology has sought to predict microbial growth by using models to describe how microbial populations grow as the conditions for growth change (Ratkowsky et al., 1983; Zwietering et al., 1994; Rosso et al., 1995; Wijtzes et al., 1995; Henk et al., 1997; see also McMeekin et al., 1993). These models assist the design of food safety systems. However, to be useful such models need to be able to predict the effects of changing external variables, such as temperature, on the growth of the microbial population. Furthermore, if risk is a consideration, the model needs to calculate the probability density of the microbial population size at any time of interest.

Generally, the effects of external variables, such as temperature or pH, on the growth of the microbial population have been modelled by considering these variables to act to change the coefficients of the chosen growth equation. However, most authors

*Corresponding author. Tel.: +64-7-838-5916; fax: +64-7-838-5117.
E-mail address: tanya.soboleva@agresearch.co.nz (T.K. Soboleva).

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have chosen to work with the integrated form of the growth equation (Zwietering et al., 1994), expressing the effect of an external variable by changes in the coefficients of the algebraic equation. The difficulty with this for modelling changes in temperature over time has been noted by van Impe et al. (1992). These authors give an example to show the errors that can arise if this formulation is used to study time-varying temperature effects.

Usually, the temperature of the product (e.g., food) can change during microbial growth. Temperature changes can be controlled, for example during food processing, or uncontrolled, for example during transport from the store to the home. If one wishes to use a growth law, such as the Gompertz equation, to describe microbial population growth, the time-dependent temperature effects need to be included in the differential equation. An advantage of this approach is that an arbitrarily complex temperature–time path can be evaluated. Thus, more realistic microbial population management can be achieved.

The nonlinear nature of most growth laws will effect the form of the probability distribution of population size as it evolves through time. Soboleva et al. (2000) showed how to calculate this probability density using a stochastic differential equation based on the Gompertz growth law. In this paper we extend this approach to include the effect of a temperature–time-dependent path on the probability (hence the risk) of the growth of a microbial population, using *Lactobacillus plantarum* as an example. Our goal is to provide a complete methodology to workers in predictive microbiology interested in applying these techniques to more variable conditions than were discussed in the previous paper. It is intended that this will assist those people interested in applying these new methods to particular situations.

2. Model formulation

The growth of a population (N) of *Lactobacillus plantarum* can be described by a stochastic Gompertz differential equation. An example based on data presented by Zwietering et al. (1994) was given recently in Soboleva et al. (2000). Defining

$$y(t) = \ln\frac{N(t)}{N_0}$$

where N_0 is the reference population size, microbial population growth is described by

$$\mathrm{d}y = ay\ln\left(\frac{y^*}{y}\right)\mathrm{d}t + \sigma y\,\mathrm{d}W \tag{1}$$

Here, a is a parameter representing the growth rate, y^* is a parameter representing the maximum population size, σ is a parameter measuring the amplitude of the noise, and $\mathrm{d}W$ is a Wiener stochastic process, i.e. a Gaussian distributed random process with zero mean and independent increments. The initial condition for Eq. (1) is given by

$$y(0) = \frac{N(0)}{N_0}$$

where $N(0)$ is the inoculation size.

Using data published in Zwietering et al. (1994) on the growth of populations of *L. plantarum*, Soboleva et al. (2000) estimated the effect of temperature on the parameters of Eq. (1). This temperature effect for the parameters a and y^* can also be derived from the work of Zwietering et al. (1994) using the equation which connects the parameter a with the specific growth rate μ in the modified form of the Gompertz:

$$a = \frac{\exp}{y^*}\mu$$

The effect of temperature on the specific growth rate of a microbial population can be described by the formulation of Ratkowsky et al. (1983):

$$\mu = (b(T - T_{\min})(1 - e^{c(T-T_{\max})}))^2 \tag{2}$$

The effect of temperature on the maximum population size y^* is given by Zwietering et al. (1994) as

$$y^* = \frac{s(T - T_{\min 1})(T - T_{\max 1})}{(T - r)(T - q)} \tag{3}$$

Zwietering et al. (1994) also consider the lag time to have independent temperature dependence. But, as shown by van Impe et al. (1992) and Soboleva et al. (2000), the lag time as defined by Zwietering et al. (1994) is a function of the initial condition to Eq. (1), growth rate and the maximum population size. Note that the initial condition of Eq. (1) includes both the initial size of the microbial population $N(0)$, and the parameter N_0. When the initial inoculation size of the microbial population is known, then for

consistent quantitative results we suggest writing the deterministic part of Eq. (1) in the form proposed by van Impe et al. (1992). This form can be used to estimate temperature dependencies directly from the data, which is the best way of dealing with this problem. In this paper the main temperature dependence in lag time λ results from the above temperature dependencies in the growth rate (a) and the log relative maximum population size (y^*) as defined by the relationship

$$\lambda = \frac{1}{a}\left[\ln\ln\left(\frac{y^*}{y(0)}\right) - 1\right]$$

Temperature T in Eqs. (2) and (3) represents the actual temperature of the product, at the site of microbial growth. This temperature follows changes in the environmental temperature $T_0(t)$ according to the Newton law of cooling:

$$\frac{dT}{dt} = \chi(T_0 - T) \tag{4}$$

The parameter χ is a constant describing the rate at which the substrate equilibrates its temperature to the change in the temperature environment. The function $T_0(t)$ is determined by the process under consideration. For example, the situation where the temperature of the environment changes abruptly from T_{00} to T_1 between times t_1 and t_2 is described by the equation

$$T_0 = T_{00} + \theta(t - t_1)\cdot\theta(t_2 - t)\cdot T_1 \tag{5}$$

The function $\theta(x)$ is a step function, equal to 1 if x is positive and zero otherwise. The sudden change in temperature described using the step function to describe the environmental temperature represents a situation where a product is removed from cold storage, transported at a higher temperature then returned to cold storage. The temperature of the product itself will change according to Eq. (4).

Eq. (5) can be modified to describe the probabilistic growth of a microbial population under any arbitrary temperature path. For example, a gradual change in temperature from T_{00} at time t_1 to T_1 at time t_2 would be given by

$$T_0 = T_{00}\cdot\theta(t_1 - t) + \frac{1}{t_2 - t_1}[T_{00}(t_2 - t_1) - T_1 \cdot (t - t_1)]\theta(t_2 - t)\theta(t - t_1) + T_1\cdot\theta(t - t_2)$$

Eqs. (1)–(5) can be combined to describe a Gompertz growth law acting under a time-dependent temperature path.

3. Results

To illustrate the procedure we apply Eqs. (1)–(4) to compare the growth of a population of *L. plantarum* under two different temperature paths. In the first case we assume that the population is continually stored at a constant temperature of 10°C for the period of interest. In this case, $T_{00} = 10$°C in Eq. (5). This is compared with a temperature path in which, after 5 h, the temperature of the environment is raised abruptly from 10 to 21°C for a period of 3 h. This could represent a situation where a product with an initial microbial population is removed from cold storage and transported at ambient temperature for 3 h before being returned to cold storage. In this case, the variable T_0 in Eq. (5) becomes

$$T_0 = 10 + \theta(t - 5)\cdot\theta(8 - t)\cdot 11 \tag{6}$$

The parameters for Eqs. (2) and (3) for *L. plantarum* given by Zwietering et al. (1994) are: $T_{\min} = 3.29$, $T_{\max} = 44.8$, $c = 0.247$, and $b = 0.0385$, $s = 10.5$, $r = 1.29$, $q = 43.7$, $T_{\min 1} = 3.29$, $T_{\max 1} = 43.1$. The parameter χ in Eqs. (1)–(4) will depend on the particular product. For illustration, this parameter is set to be equal to 1. The initial log population size was chosen to be 0.1. Fig. 1 shows (for clarity) the deterministic growth (meaning $\sigma = 0$) of two populations of microbes, one remaining at 10°C while the other undergoes the temperature shift described above. The growth of the microbial population subject to a period of higher temperature is obviously faster. Eventually, both populations will reach the same maximum population (not shown).

Microbial population growth with a temperature shift is a stochastic process described by the set of equations (1) to (4) with Eq. (5). The probability distribution of log population size over time is calculated by combining 50,000 simulations of this process. The additional parameter required, namely the process variance, is assumed to be $\sigma = 0.0425$ from estimates made by Soboleva et al. (2000) on *L. plantarum*.

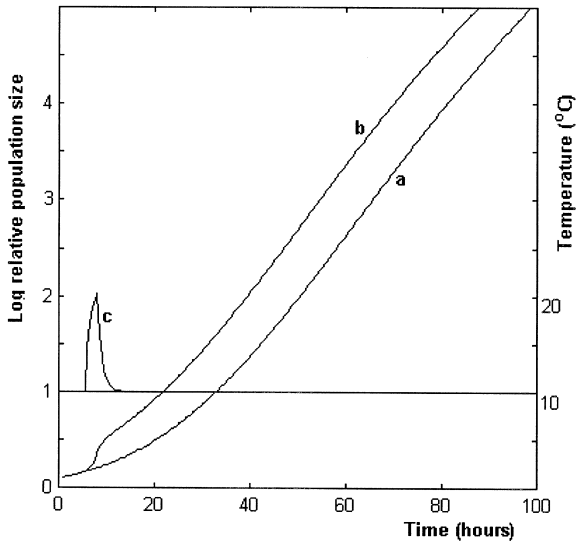

Fig. 1. The deterministic growth in the log relative population size of *L. plantarum* under two temperature pathways. (a) Population group grows at a constant temperature of 10°C. (b) Population group experiences a sudden increase in environmental temperature of 11°C between the times of 5 and 8 h. (c) Time–temperature profile $T(t)$, representing the solution of Eq. (4).

Fig. 2 compares the probability distributions of the log population sizes of the microbes kept at 10°C (Fig. 2a) against the probability distributions of the microbes undergoing the temperature shift (Fig. 2b). In Fig. 2 the probability distributions are shown for each 15-h time difference. Initially, both bacteria populations had the same probability distributions. The higher mean log population size, and the greater variance of the log population size (loosely measured by the extent of the distribution peaks) of the subsequent probability densities of the microbes undergoing the temperature increase are apparent. Furthermore, at $t = 45$ the population without the temperature shift has about zero probability of reaching a log relative population size of 2.5. However, the population experiencing an increase in temperature has a log population mean of about 2.5 at this time.

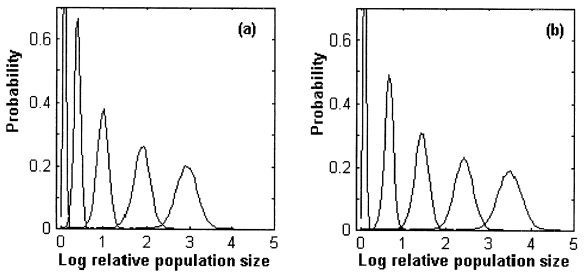

Fig. 2. The evolution of the probability distributions of log relative population sizes through time for (a) the *L. plantarum* population maintained at a constant temperature of 10°C and (b) the *Lactobacillus* population experiencing a temperature increase from 10 to 21°C at 5 h. The probability distributions are shown for times 0, 15, 30, 45 and 60 h. In each case the initial log relative population size was 0.1 with a standard deviation of 0.025.

Fig. 3 illustrates the application of the methodology of Soboleva et al. (2000) to calculating, for each population, the probability that the log population will exceed a critical size given an initial population size. For illustration, the critical population size is chosen to be $y = 4$. Even though the temperature increase took place at a time of 5 h this does not significantly increase the probability of exceeding the critical size ($P < 0.1$) until about 50 h has elapsed. However, for the microbial population maintained constantly at 10°C there is no obvious effect until about 68 h has elapsed. In this case, at a time of 75 h, the probability of exceeding the critical population size is 0.18 for the microbes held at 10°C, but 0.98 for the microbes experiencing the earlier temperature increase.

Fig. 4 compares the response of the probability in exceeding the critical log population size of 4 at time 75 h due to the size of the temperature shift occurring at time 5. The smaller the temperature change, the lower the probability of exceeding this critical value. Fig. 5 shows the effect of the duration of the temperature change at time 5 h on the probability of exceeding the critical log population size of 4 at time 75 h. Similarly, the shorter the time the temperature change is applied, the lower the probability of exceeding the critical value.

An application of the above concepts to the management of food safety is given in Fig. 6. This figure shows the size of the initial log population necessary if the critical log population size of 4 is to be reached with a probability of 10% within a nominated time for each temperature regime. And vice versa, knowledge of the initial log population allows estimating the critical time, i.e. time when the log of the population size reaches its critical value.

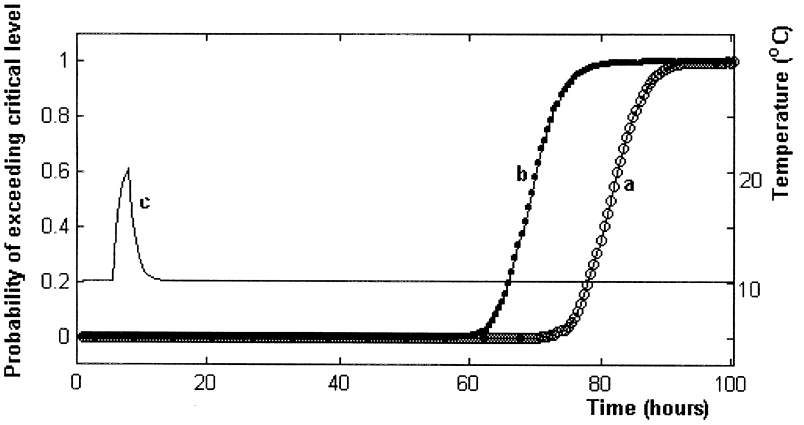

Fig. 3. The probability of exceeding a critical level, chosen to be a log relative population size of 4, depending on time, if the initial log relative population size is 0.1. (a) *L. plantarum* growing at a constant temperature of 10°C. (b) *L. plantarum* growing under the temperature profile shown in (c).

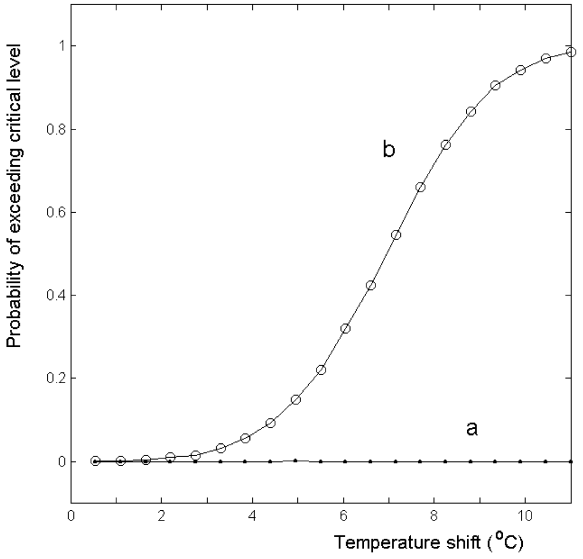

Fig. 4. The probability of exceeding a critical level, chosen to be a log relative population size of 4, at 75 h depending on the maximum of the temperature increase which begins at 5 h. (a) Control population growing at a constant temperature. (b) Population experiencing a change in temperature.

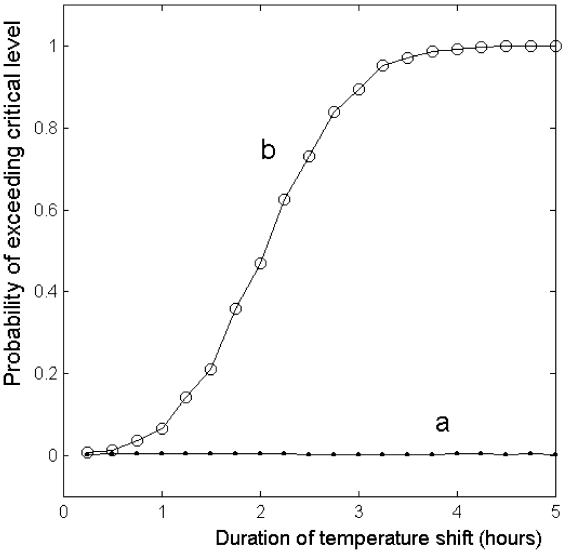

Fig. 5. The probability of exceeding a critical level, chosen to be a log relative population size of 4, at 75 h depending on the duration of the temperature increase which begins at 5 h. (a) Control population growing at a constant temperature. (b) Population experiencing a change in temperature at 5 h.

4. Discussion

In this paper we have examined only the temperature range where *L. plantarum* exhibits normal growth. It is obvious that if the temperature rises too high the microbes will be killed, as reported by Smith and Marmer (1991). Eq. (3) does not incorporate this aspect of microbial physiology.

This paper extends the methods of previous work

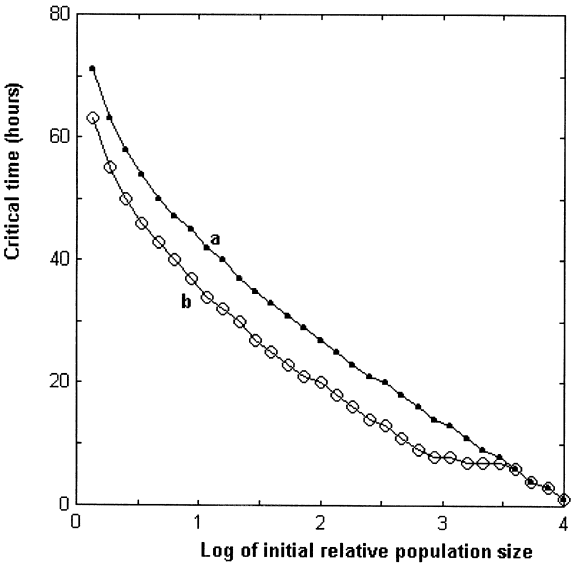

Fig. 6. The time taken to reach a log relative population size of 4 with a probability of 10% given the size of the initial log relative population size. (a) Population growing at a constant temperature. (b) Population experiencing a change in temperature at 5 h.

(Soboleva et al., 2000) by including the effect of a time-dependent external variable in the calculations of the probability densities of microbial population growth. The view of van Impe et al. (1992) that the effects of time-dependent external variables, such as temperature, need to be calculated by incorporating these variables into the differential equation is endorsed, especially when the probability densities are to be calculated as in this case. Although the set of equations (1)–(5) cannot be solved explicitly, this need not be a disadvantage. Numerical solutions are quite satisfactory for practical applications, and the procedure can be easily packaged into a decision support routine tailored for particular problems.

In this example we have used temperature as the only time-dependent external variable affecting the growth of the population of microbes. However, the method can cope with any number of time-dependent external variables and their interactions with each other. The pH of the growth medium may also vary with time and is known to affect microbial growth. This relationship can be included in Eq. (4) in a straightforward way, and the results calculated.

There is a role for this formulation in identifying HACCP points along the supply chain. At these points a measurement of microbial contamination is taken and a decision made whether to retain the product in the supply chain or not. The basis of this decision is the likelihood that the product will be unacceptably contaminated at the point of consumption, even though it is below tolerance limits at the HACCP point of measurement. This means that the probability that the product will become unacceptable at some time in the future needs to be calculated, given the likely environment of external variables such as temperature.

However, this calculation is the same as that illustrated in Figs. 3, 4 and 5. The basis for automatic evaluation is shown in the example of Fig. 6. In this case, if the food safety protocol dictated that the probability of reaching a critical log relative population size of 4 is less than or equal to 10%, then the log relative population size should not exceed the sizes shown earlier than the indicated critical time. If this happens the product should be rejected.

To turn this formulation into a practical tool will require consideration of the sampling error at the point of measurement. This information can then be combined with the above theory to construct a suitable system for monitoring food safety along a supply chain. For example, a cusum chart (Murdoch, 1979) can be constructed based on this information.

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