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Stochastic modelling of bacterial lag phase

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Abstract

In order to study the lag distribution of the individual cells in a bacterial population, a stochastic birth model is used in this study. An integral formula is applied to transform the assumed lag distribution into a growth function describing the transition between lag and exponential phase of the cell population. By means of this formula, it is pointed out that traditional viable count curves are not suitable to identify the distribution of individual cells' lag time. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Bacterial growth; Bacterial lag; Distribution of lag times; Stochastic birth process

1. Introduction

Predictive microbiology models have so far dealt predominantly with the specific growth rates (or, equivalently, doubling times) of various organisms in different environments characterized primarily by some factors, such as temperature, pH, available water, etc. (McMeekin et al., 1993). When lag times are modelled, the predictions are much poorer. The reasons are analysed, for example, in Baranyi and Roberts (1995).

Renshaw (1991) emphasized that deterministic models are not suitable to predict population dynamics at low counts and stochastic models should be used for that situation. Appropriate stochastic birth–death models for long have been applied to biotechnology applications (Tsuchiya et al., 1966; Frederickson et al., 1967) and medical studies (Armitage et al., 1965). In predictive food microbiology, the demand to predict probabilities of survival and growth at low cell numbers began to increase only recently, with the need for quantitative risk assessment applications. More recently, Hills and Wright (1995), Baranyi (1998) and Baranyi and Pin (1999) have applied stochastic mathematical models for predictive microbiology studies.

Information on the distribution of the lag times of individual cells is important from risk assessment point of view because the "rare" cells

(i) can unexpectedly shorten the population lag time;
(ii) can reflect the history of the cells (such as food processing conditions);
(iii) are paramount to establish the probability of survival, recovery and growth in a future environment (such as storage).

This distribution is not the same as that obtained from traditional growth curve replicates where the lag times are estimated from fitted growth functions. The variance obtained in that way would be the

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variance of the population lag, characterising rather the randomness of the environment and the measurement method, so the variance of the lag times of the individual cells would disappear in the 'noise'. The traditional technique of fitting deterministic models to growth/death curves, as will be demonstrated in this paper, is unable to handle the variability of the cells. A different approach, based on the analysis of the distribution of the individual lag times, is necessary to model the probability of survival and growth more accurately at low counts.

2. Materials and methods

2.1. A stochastic birth process model

The time-dependent size of a growing bacterial cell population, denoted by $x(t)$, is commonly represented in log-scale against time, in which case, the lag and the exponential phase form a J-shaped curve (Fig. 1). If $y(t)$ denotes the natural logarithm of the cell counts, the $y(t)=\ln x(t)$ curve is approaching to the $y=\mu(t-L)$ linear function, where μ is called the specific growth rate. That parameter can also be interpreted as the increase of the population in a unit time per the number of cells producing that increase. Following the terminology introduced in Baranyi (1998), the delay parameter L is called population lag as opposed to the lag times of individual cells. In what follows, the J-shaped curve is meant as 'growth curve'. Generally, a growth curve is completed also with a stationary phase, lending itself to a sigmoid shape, with a straight segment around the inflexion. In this paper, however, we consider only the lag and the exponential phase, and, for the sake of simplicity, we model them by a J-shaped curve.

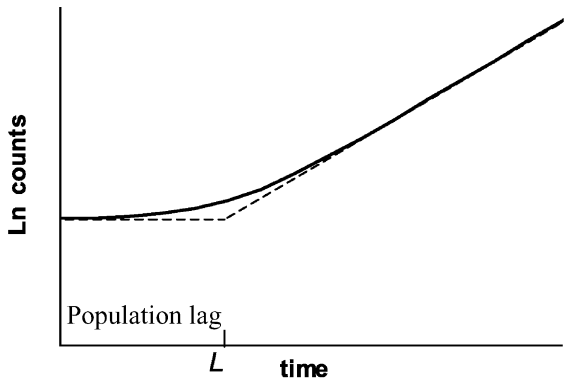

Fig. 1. J-shaped growth curve (thick line) of the natural logarithm of cells against time. In the exponential phase, the curve converges to a delayed linear function (broken line) where the delay is L, the population lag.

Let the initial number of a growing cell population be N. Suppose that the population is homogeneous, i.e. the lag times of the individual cells, denoted by τ_i ($i=1, 2, \ldots, N$) are identically distributed independent random variables, with an expected value $E(\tau_i)=\tau$.

We assume that once a cell divided, the subsequent daughter cells will be in exponential phase, i.e., at the time t, the expected size of the subpopulation generated by the i-th cell is

$$x_i = \mathrm{e}^{\mu \max(0, t-\tau_i)}$$

Finally, suppose that the subsequent subpopulations of each cell of the initial culture grow together, but independently of each other (for example, the cells are far enough from each other that we can neglect any possible ''communication effect'' or ''quorum sense'').

3. Results and discussion

By some algebra with conditional probabilities, it can be proven that, at the time t, the expected size of the population starting from N cells is

$$x(t) = \sum_{i=1}^{N} x_i(t) = N\left(\int_0^t \mathrm{e}^{\mu(t-s)} f(s)\mathrm{d}s + \int_t^\infty f(s)\mathrm{d}s \right) \quad (1)$$

where $f(t)$ denotes the (common) probability density function of the individual lag times, τ_i ($i=1, 2, \ldots, N$) (see Baranyi and Pin, 2001).

Take the logarithm of the above equation:

$$y(t) = y_0 + \mu t + \ln\left(\mathrm{e}^{-\mu t}(1 - F(t)) + \int_0^t \mathrm{e}^{-\mu s} f(s)\mathrm{d}s \right) \quad (2)$$

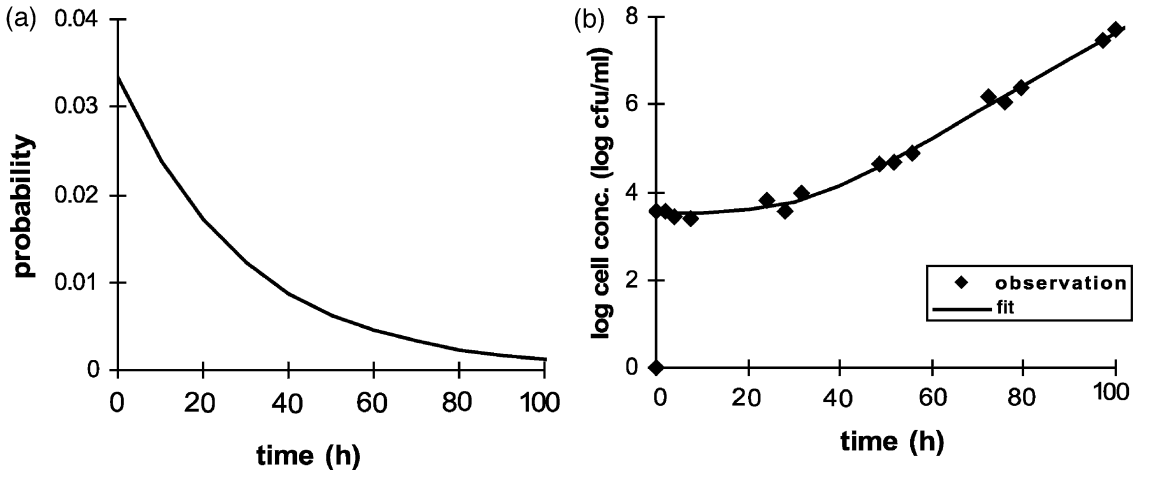

Fig. 2. One-to-one mapping between (a) the probability density function of the exponential distribution assumed for the individual lag times and (b) the growth curve characterizing the transition of the population from the lag to the exponential phase.

where $F(t)$ is the cumulative distribution function of the individual lag times and $y_0 = \ln N$.

The above formula describes a one-to-one mapping between the distribution of individual lag times and the shape of the growth curve of the population (see Fig. 2). The growth curve converges to a straight line as t approaches infinity (Baranyi and Pin, 2001), so (not surprisingly) it is the curvature from the lag to the exponential phase that characterizes the wanted distribution. This is similar to that situation when, in inactivation models, a Laplace transformation maps the distribution of resistance of individual cells onto a survival curve (Körmendy et al., 1998).

By Eq. (1), if one could observe the lag times of individual cells and one or two subsequent divisions in the exponential phase (to estimate μ), the growth

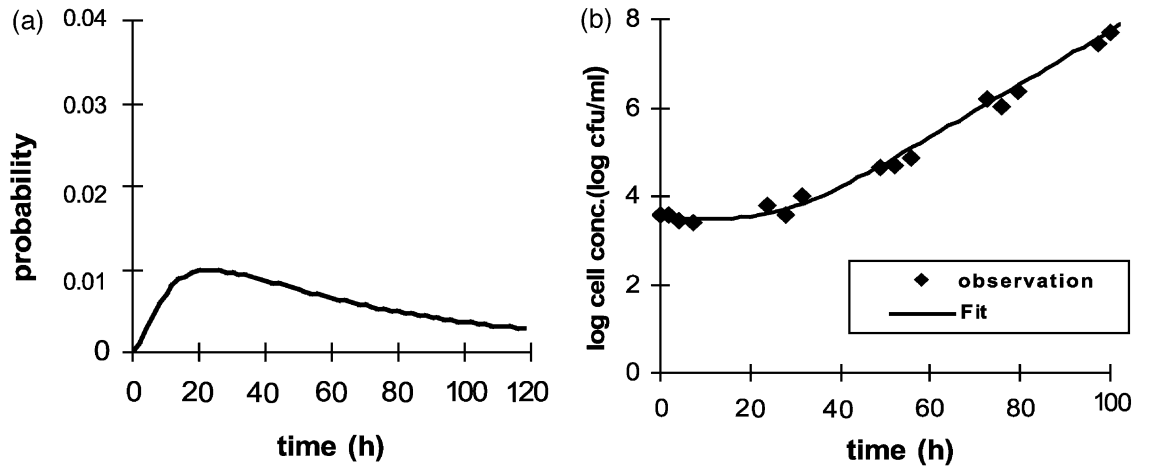

Fig. 3. As in Fig. 2, but lognormal assumption for the distribution of the individual lag times. The two distributions (Fig. 2a and panel (a) of this figure) are markedly different, still the respective two population growth curves (Fig. 2b and panel (b) of this figure) are indistinguishable from each other.

curve of the whole population could be predicted. In the opposite direction, it should be also possible to estimate the distribution of the lag times of individual cells from traditional viable count growth curves, if there is sufficient amount of accurate measurements before the exponential phase.

However, Eq. (1) is a one-to-one mapping only in theory. In practice, it is not feasible to identify the distribution of the individual lag times from traditional viable count growth curves.

For an example, consider the growth curve on Fig. 2b, which is one of the growth curves in the paper of McClure et al. (1993). Test the exponential distribution for $f(t)$. Let it be

$$f(t) = v\mathrm{e}^{-vt}$$

where v is the reciprocal of the average lag time of the individual cells: $v = 1/\tau$. Our Eq. (1) reads as follows:

$$y(t) = y_0 + \mu t + \ln\left(\frac{v}{\mu+\nu} + \frac{\mu}{\mu+\nu}\mathrm{e}^{-(\mu+v)t}\right)$$

After converting the raw data into natural logarithm of the cell concentrations, the three parameters, y_0, μ, v, can be fitted by least squares method. The fitted curve, after transforming it back to $\log_{10}$ scale, can be seen in Fig. 2b.

Though the fit is obviously good, one must note that this is the best fit if $f(t)$ is chosen from the family of exponential distributions for the individual lag times. Indistinguishably similar fits can be obtained if $f(t)$ describes gamma (of which exponential is only a special case) or lognormal distribution (Fig. 3).

It must be realized that, though Eq. (1) provides a theoretical background to make equivalence between growth curves and lag distributions, the standard curve fitting procedures, numerically, are not robust enough on commonly measured growth data. The traditional viable count curves are not suitable to identify what type of distribution is followed by individual cells. New approaches are necessary in the measurement techniques, too (image analysis, flow cytometry, etc.), in order to study individual cell kinetics.

Deterministic models can be obtained from stochastic ones but the relation is not reversible. This underlines the need to develop stochastic models for the dynamics of individual cells, in order to predict bacterial lag, growth and survival more accurately.

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