

# Estimating Risk from Small Inocula by Using Population Growth Parameters<sup>∇</sup>

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**Risk from an uncertain small inoculum depends on variability of single-cell lag times. However, quantifying single-cell variability is technically challenging. It is possible to estimate this variability using population growth parameters. We demonstrate this possibility using data from literature and show a Bayesian scheme for performing this task.**

An inoculum size effect on a bacterial population lag phase has been demonstrated in many studies of bacterial growth (2, 7, 9). These authors showed that, with smaller inoculum levels, the uncertainty in the population lag parameter increases. Pin and Baranyi (8), using a computer simulation model, demonstrated that the inoculum effect on the population lag time was not evident when more than 40 cells were used to initiate growth in their system.

A bacterial population at time  $t$ , grown from an inoculum consisting of  $n$  cells, can be represented by

$$N(t) = \sum_{i=1}^n e^{\mu \times \max(t - L_i, 0)} \quad (1)$$

where  $\mu$  is the specific growth rate for cells (we assume this is constant within the cell inoculum). We assume that the lag times of individual cells in the inoculum,  $L_i$ , are identically and

independently distributed random variables. Taking the natural logarithm of the cell population in equation 1, for sufficiently large time  $t$ , and comparing the result with a biphasic model for growth gives the population lag time,  $\lambda$ , arising from an initial inoculum of size  $n$  as (see, e.g., reference 5)

$$\lambda = -\frac{1}{\mu} \ln \frac{\sum_{i=1}^n e^{-\mu \times L_i}}{n} \quad (2)$$

In this report we will show that this model is consistent with actual observations of growth from small inocula and therefore that it can be used, in combination with easily obtainable population parameters and Bayesian inference, to estimate details of single-cell variability. This approach is in contrast to using observations from experiments initiated with small inocula, such as a bioscreen, to infer single-cell variability parameters

TABLE 1. *Listeria monocytogenes* growth data from the ComBase database with equivalent single-cell growth parameters of Francois et al. (4) and the estimated population parameters  $\lambda^a$

Identification code(s) for growth data from ComBase	Environmental condition		Distribution (single-cell lag time variability) <sup>b</sup>	Mean single-cell lag time (h)	Estimated population lag $\lambda$ (h)
	Temp (°C)	pH			
DuhLm_17 and DuhLm_18	30	7.4	Gamma (1.20, 0.65)	0.8	0.6
ADRIAN_31, B113_51, and DuhLm_9	10	7.4	Gamma (1.28, 6.40)	8.2	5.7
B113_49, DuhLm_7, DuhLm_8, ENVA52, ENVA65, ENVA69, and ENVA74	7	7.4	Gamma (1.11, 9.07)	10.1	7.5
ENVA50 and ENVA61	4	7.4	Gamma (2.09, 19.23)	40.2	28.4
B166_12, B166_13, LM080_1, and LM082_1	10	6.1	Weibull (2.66, 31.01)	27.6	19.9
B166_19, B166_20, ENVA91, Laug_14a, Laug_14c, and Laug_160a	7	6.1	Weibull (3.68, 79.24)	71.4	51.2
B288_2 and B288_113	4	6.1	Weibull (2.01, 91.04)	80.7	54.7
B166_11	10	5.5	Weibull (2.93, 34.08), shift of 24.37	54.8	46.6
B166_18 and B165_18	7	5.5	Weibull (4.29, 119.4)	108.7	70.74
B288_14, B288_131, and B428_110	4	5.5	Weibull (3.04, 187.1)	167.2	119.3

<sup>a</sup> *Listeria monocytogenes* growth data from the ComBase database (www.combase.cc). The water activity  $a_w$  is 0.997 or equivalently, 5% NaCl.

<sup>b</sup> Numbers in parentheses are the parameters of the probability distributions.

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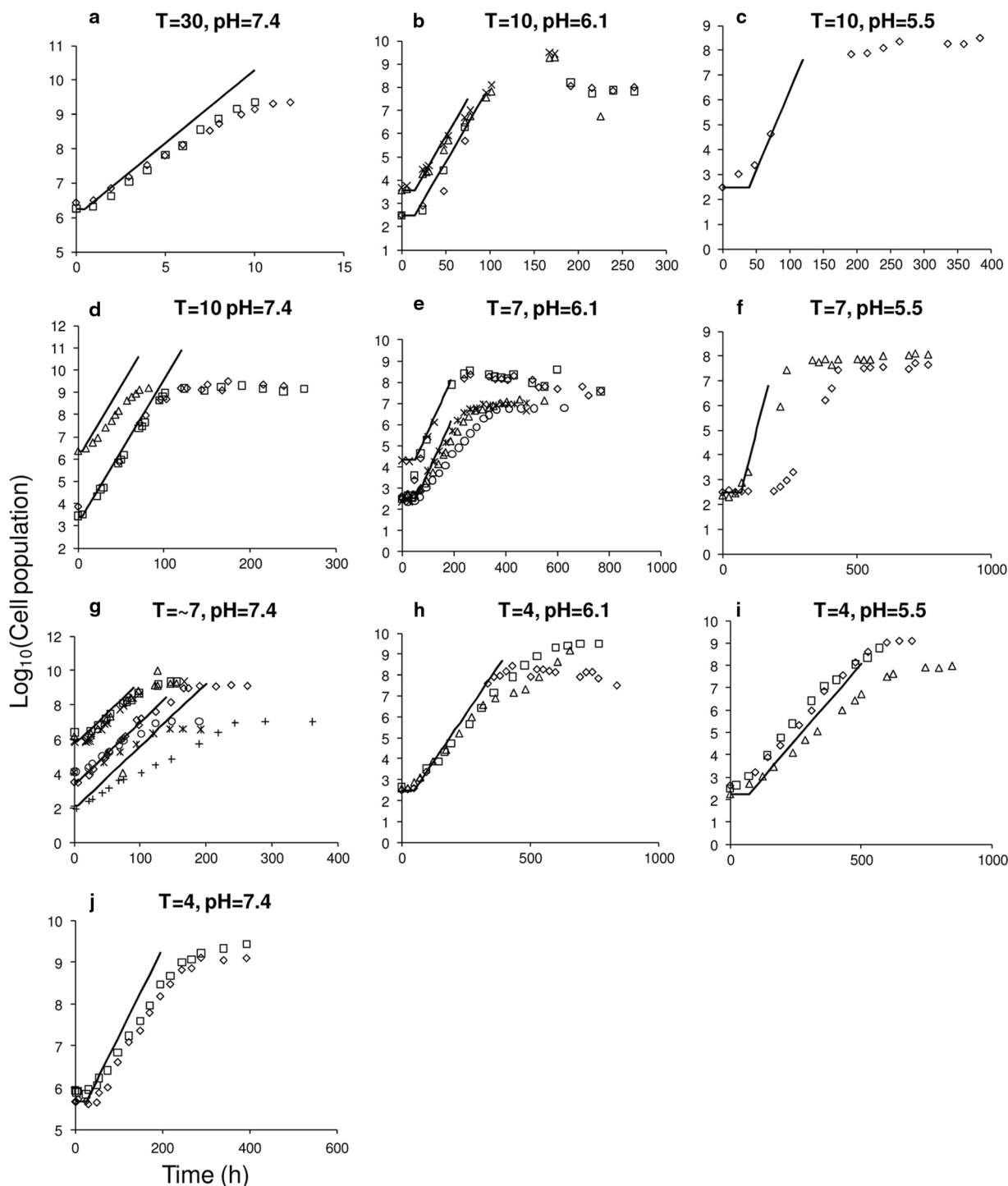


FIG. 1. Comparison of modeled data (solid line) derived from the single-cell growth parameters of Francois et al. (4) and experimental growth data for *Listeria monocytogenes* from ComBase database (www.combase.cc). For all graphs, the y axis is  $\log_{10}$  (cell population) and the x axis is time (in hours).

(see, e.g., reference 5) and hence is valuable when these measurements are unavailable.

Francois et al. (4) measured a comprehensive set of individual cell lag times for *Listeria monocytogenes* under different environmental conditions and quantified the variability of these lag times using either a gamma or Weibull distribution.

We have used these variability distributions of single-cell lag phase to derive a population lag,  $\lambda$ , and compared modeled growth data to experimental growth data from the ComBase database (www.combase.cc). The ComBase database currently contains about 9,000 *L. monocytogenes* data records. We selected 32 growth curves from ComBase which closely matched the en-

vironmental conditions used by Francois et al. (4). Table 1 gives the database identification numbers of the *L. monocytogenes* growth data used in this study, the corresponding single-cell growth parameters of Francois et al. (4), and the derived population lag for  $n = 10^5$ .

Figure 1 shows a comparison of the modeled growth, estimated using  $\lambda$  and the generation times from Francois et al. (4), with growth data from ComBase. Comparatively,  $\lambda$  is of the same order of magnitude as the population lag phase from a fit using a trilinear model (3), with the exception of growth at pH 5.5 (Fig. 1c, f, and i). In Fig. 1c, growth at 10°C and pH 5.5, the fitted population lag phase is  $\sim 7$  h, much shorter than at 10°C and pH 6.1, which is counterintuitive. We suspect that the growth data have been mislabeled in Fig. 1f. The growth data shown in Fig. 1f compare growth of heat-injured cells and normal cells under the same conditions. The growth data showing a shorter population lag is labeled as growth from heat-injured cells. In Fig. 1i,  $\lambda$  is approximately double the fitted population lag phase. We are unable to resolve this discrepancy, and further investigation is warranted. However, given the diverse information sources and the uncertainties in the estimation of the population lag phase, the convergence between modeled and experimental data is very good.

It is not possible to resolve individual lag phases given  $\lambda$  from equation 2. However, it is possible to use the central limit theorem to derive individual lag phases using a Bayesian scheme. We will use the following example to demonstrate this scheme, where the lag phases of individual cells,  $L$ , follow an exponential distribution, with expectation  $\tau$  and variance  $\tau^2$ . In the limit of large  $n$ , the expected value of  $\lambda$  is

$$E(\lambda) = \frac{\ln(1 + \mu\tau)}{\mu} \quad (3)$$

and the variance of  $\lambda$  is

$$\sigma^2(\lambda) = \frac{1}{n} \left( \frac{1 + \mu\tau}{\mu} \right)^2 \left( \frac{1}{1 + 2\mu\tau} - \left( \frac{1}{1 + \mu\tau} \right)^2 \right) \quad (4)$$

(see, e.g., reference 1). We may then express the conditional probability as

$$p(\lambda|\tau, \mu, n) \sim \text{Normal} \left( \frac{\ln(1 + \mu\tau)}{\mu}, \frac{1}{n} \left( \frac{1 + \mu\tau}{\mu} \right)^2 \left( \frac{1}{1 + 2\mu\tau} - \left( \frac{1}{1 + \mu\tau} \right)^2 \right) \right) \quad (5)$$

which will be a good approximation for sufficiently large  $n$  (typically an  $n$  of  $\sim 100$  is large but, in some cases, larger values are required for convergence).

Bayes' theorem can then be used to infer the single-cell lag phase variability given appropriate prior information on  $\tau$  and  $\mu$  and experimental evidence about  $\lambda$  (and on  $\sigma_n$  [the standard deviation of the normal distribution of the population lag for  $n$  cells] if data are available). The following case study involves the growth of *Escherichia coli* at 20°C in a tryptic soy broth culture. Niven et al. (6) conducted studies of single-cell *E. coli* growth using a digital-image analysis technique. The mean time to the first division of single cells in their system was  $\sim 2.5$  h. The closest match of this growth condition for *E. coli* in the ComBase database are the growth curves identified by

Tas1234, Tas1235, Tas1236, Tas1237, and Tas1238 (which in this report we consider a broad homogeneous population). The population lag  $\lambda$  (h) and the specific growth rate  $\mu$  ( $\text{h}^{-1}$ ) for Tas1234, Tas1235, Tas1236, Tas1237, and Tas1238 obtained from a biphasic fitting procedure (www.combase.cc), are 4.5 and 0.34, 2.2 and 0.30, 3.0 and 0.29, 0.9 and 0.32, and 1.8 and 0.28, respectively. Using prior information for  $\tau$ , specified by a uniform distribution in the range 0 to 10 h, and the population growth parameters and applying Bayes' theorem to equation 5, results in a posterior expectation of  $\tau = 2.1$  h (similar results might be obtained by using alternative asymmetric distributions to represent the variability of single-cell lag times).

Barker et al. (2) showed a Bayesian scheme for estimating individual germination parameters of spores of nonproteolytic *Clostridium botulinum* from population growth data and validated their data using data from Webb et al. (M. D. Webb, S. C. Stringer, R. B. Piggott, J. Baranyi, and M. W. Peck, presented at the 2nd International Conference on Analysis of Microbial Cells at the Single Cell Level, Vejle, Denmark, June 2002). This scheme can be generalized to other parameterizations of single-cell variability, e.g., the gamma and Weibull distribution. Currently, there are no studies done using this scheme to infer single-cell lag phase variability.

Single-cell lag phase variability plays an important role in calculating risk because good manufacturing practice and hygienic production methods invariably reduce bacterial loads in manufactured foods. If only the expected value of  $\lambda$  is used for addressing safety, then the chance of underestimating risk due to cells which have short lag phases increases. Since it is technically challenging to estimate single-cell variability, the Bayesian scheme we have introduced in this article provides a method for estimating this variability using an established and accessible experimental protocol.

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