A novel approach for estimating the lag phase duration of food-borne pathogens

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Introduction

The presence of pathogenic bacteria in foods presents a serious hazard to consumers, and is of great concern to the food industry. End-product testing is expensive and labour-intensive, thus alternative approaches to the control of food-borne pathogens are sought. In support of these efforts, the field of predictive microbiology -the application of mathematical models to microbial survival and growth- has expanded in recent years, with two books having been published within the last decade (3, 5).

When bacteria are exposed to a favourable environment, growth generally follows a sigmoidal curve (Figure 1). The initial phase -the lag- is a period in which the cells become adapted to the new environment, followed by a phase of exponential growth. The stationary phase results from the accumulation of toxic waste products or the depletion of essential nutrients, and is sometimes followed by a death phase. The lag and exponential growth phases are of most interest to food microbiologists, because it is during these periods that microbes are most sensitive to intervention strategies.

In order to predict the growth of bacteria it is necessary to calculate the lag phase duration and the exponential growth rate from sigmoidal growth curves, and various models have been proposed (4). The growth rate is generally easy to estimate, since it depends solely on the current environment; however, the lag phase is more challenging, since it is largely influenced by the prior history (or physiological state) of the inoculum. Figure 2 shows the lag phase in more detail. The lag is defined as the point relative to the time axis at which a tangent to the exponential portion of the growth curve (line "a") -with slope equal to the growth rate mmax- intersects the intial cell number (N0; line "b"). The parameter h0 is the physiological state, representing the readiness of the population for growth, and defines the relationship between the lag and growth rate: h0=Lag? mmax.

Fitting sigmoidal growth models requires extensive data over most or all of the three growth phases. Often only limited data are available from the early stages of growth, thus it would be useful to have a simple method for estimating the lag time.

Development of the model

From Figure 2 we can see that, at the end of the lag phase, the total number of cells (represented by the solid line) has increased over the initial cell number, N0. The magnitude of this increase can be calculated by making some assumptions about the cell population. We assume that, at the start of the experiment, there are two populations of cells: those that start growing at t=0 with no lag (G), and those that do not grow (NG). The G cells grow exponentially from an initial number (G0), and give rise to line "a" in Figure 2 (note that the Y-axis is scaled in natural logarithm [ln] units). The total number of NG cells at t=0 is N0-G0, and does not change with time. Adding these populations together at each time interval gives the solid line in Figure 2, the growth curve.

At the end of the lag phase (where lines "a" and "b" intersect), the NG cells are unchanged, while the G cells have grown from G0 to a number equivalent to the intial cell number, N0. Thus the total cell population at t=Lag (NLag) is 2N0-G0. It is now possible to see that the NLag changes in a predictable manner. If the value of G0 is small (resulting in a long lag), then NLag approaches 2N0. When the lag phase is short, or absent (as in the case of exponential cells), most or all of the cells are initially G0, and NLag=N0. Thus the total number of cells at the end of the lag phase is between N0 and 2N0. Generally, unless the lag is very short, the value of NLag is close to 2N0 (although the mathematics involved in demonstrating this is rather complex, and will not be presented here).

Interpretation

We have limited knowledge of the actual behaviour of cells during the lag phase, so the model described above is based on some assumptions. However, the
intercept of lines "a" and "b" is generally accepted as the definition of the lag, and the mathematical representation presented here is based on accepted growth models (1, 2). We can now define the lag phase as the time required for the initial cell population to double. Thus, in cases where there are limited data over the whole growth period, it is sufficient to have data from the first few hours of growth. Using a non-linear regression program, curves such as a cubic spline can be fitted to the data, and through interpolation it is possible to calculate the time required for the initial cell population to double, and get a good estimate of the lag time.

The lag time is a concept related to the bacterial growth curve, but in fact it has no real meaning in terms of the potential risk posed by a culture of bacteria. The lag is often thought of as a "safe" period; however, we have seen that over this period the initial cell population can double. If we consider that a doubling of the population is a simple measure of the potential risk, we see that, in the case where the lag is long, a doubling occurs by the end of the lag phase. Where there is little or no lag, a doubling of the cell number is simply the doubling time of an exponential population. Thus, the time required for a food-borne pathogen to double in number can be used as a simple measure of bacterial lag time, and an estimate of the risk.

References

1. **Baranyi, J., T.A. Roberts, and P. McClure**. 1993. A non- autonomousdifferential equation to model bacterial growth. Food Microbiol. 10: 43-59.

2. **McKellar, R.C.** 1997. A heterogeneous population model for the analysis of bacterial growth kinetics. Int.J.Food Microbiol. 36:179-186.

3. **McKellar, R.C. and X. Lu**. 2003. Modeling microbial responses in food. CRC Press Inc., 2000 Corporate Blvd NW/Boca Raton/FL 33431/ USA.

4. **McKellar, R.C. and X. Lu**. 2003. Primary Models, p. 21-62. In R.C. McKellar and X. Lu. (ed.), Modeling microbial responses in food. CRC Press Inc., 2000 Corporate Blvd NW/Boca Raton/FL 33431/USA.

5. **McMeekin, T.A., J.N. Olley, T. Ross, and D. A. Ratkowsky** . 1993. Predictive microbiology: Theory and application. John Wiley & Sons Inc., New York.

Figure 1. Bacterial growth curve

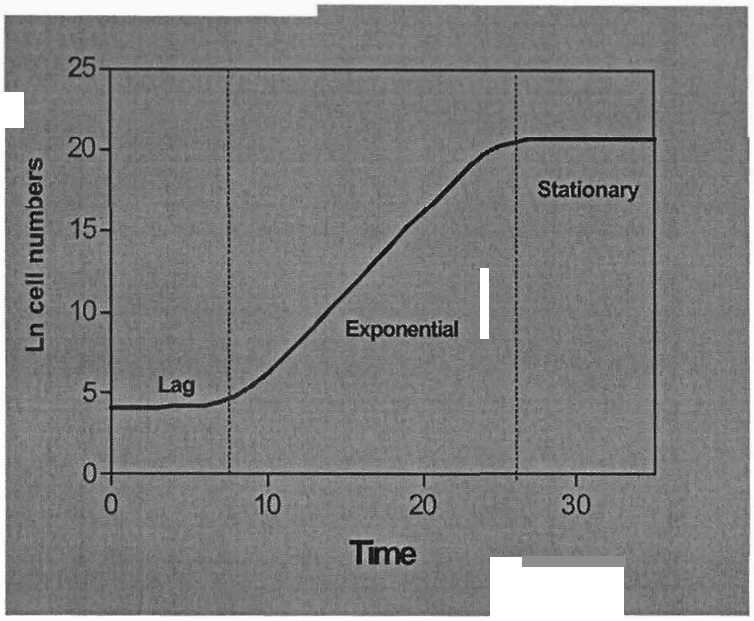

Figure 2. Mathematical parameters describing the lag phase of the bacterial growth curve