Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Food Microbiology 106 (2006) 169 – 175
INTERNATIONAL JOURNAL OF Food Microbiology
www.elsevier.com/locate/ijfoodmicro

Assessment of distributions for fitting lag times of individual cells in bacterial populations ☆

Robin C. McKellar *, Aileen Hawke

Food Research Program, Agriculture and Agri-Food Canada, 93 Stone Road West, Guelph, ON, Canada N1G 5C9

Received 24 January 2005; received in revised form 16 May 2005; accepted 30 June 2005

Abstract

To develop mathematical models describing lag times of individual bacterial cells (τ), experimental τ data were fitted to a variety of continuous distributions using BestFit. Six strains of *Escherichia coli* O157:H7 were used, and serial dilutions were made in Bioscreen multi-well plates to get single cells per well. Detection times (t_d) for individual wells were converted to τ using the maximum specific growth rate (μ) for each strain. All strains were subject to 25 trials, with up to 100 replicate wells per trial. Some strains had significantly longer t_d, and lower μ, but the τ values were not significantly different. Distributions were best fit in the order Pearson V > Pearson VI > Extreme Value > Lognormal > Lognormal2 > Inverse Gaussian based on the Anderson–Darling statistic. The Lognormal distribution was selected because there was less variability in the parameter values, and parameters have specific biological meanings. Distributions could be fit to sample populations as low as six, with fittings and parameter values comparable to those obtained with larger samples (up to 89). Extreme Value, Pearson V, and Pearson VI distributions were more suitable for fitting τ values generated from a Lognormal distribution when the numbers of sample points were few, which suggested that there are similarities between the distributions. The results suggest that a Lognormal distribution can be used successfully to characterize τ.

Keywords: *Escherichia coli* O157:H7; Lag; Growth; Distributions; Lognormal; Mathematical models; Predictive microbiology

1. Introduction

Modelling the survival and growth of spoilage microorganisms and pathogens in foods has developed into an important field of food microbiology (McMeekin et al., 1993; McKellar and Lu, 2003a). The ability to model both the adaptation of a microorganism to a new environment and its subsequent growth is particularly important for understanding the behaviours of pathogens in foods. Modelling the lag phase, i.e. the transition from the stationary or resting phase to exponential growth, presents the greatest challenge. In an attempt to understand this process, several deterministic models utilizing adjustment functions or compartments have been proposed (Baranyi et al., 1993; Hills and Wright, 1994; McKellar, 1997). These models present advantages over the more traditional curve fitting approaches, and have been the subject of recent reviews (McKellar and Lu, 2003b; Baty and Delignette-Muller, 2004). One interesting aspect of these models is that, while they are based on different biological hypotheses, they are mathematically very similar (Baty and Delignette-Muller, 2004).

It is becoming clear that to develop a more complete understanding of the lag phase process, the behaviour of single cells has to be taken into account through the development of stochastic models (Baranyi and Pin, 2003). Models have been developed which not only relate the distribution of individual cell physiological states or lag times (τ) to population lag (λ) (Baranyi, 1998), but which also have included a transformation of τ distribution into a growth function (Baranyi, 2002). In another approach, adaptation was simulated as a series of discrete events coupled with a growth function (McKellar and Knight, 2000; McKellar, 2001). A key step for improving models is to relate the physiological state of cells to behavior; however, it is difficult to get information on τ values.

There are a number of techniques which can be used to study τ values. The most common is the turbidimetric method, in which serial dilutions of cells are made in multi-well plates to eventually attain single cells per well (Stephens et al., 1997; McKellar and Knight, 2000). Time-to-detection information

☆ Contribution no. S210 from the Food Research Program.

* Corresponding author. Tel.: +1 519 780 8026; fax: +1 519 829 2600.
E-mail address: McKellarR@agr.gc.ca (R.C. McKellar).

0168-1605/$ - see front matter.
doi:10.1016/j.ijfoodmicro.2005.06.018

can be gathered, and used to calculate τ. Alternatively, single-cell division can be observed microscopically, although this is technically difficult (Wu et al., 2000; Elfwing et al., 2004). Comparison of the areas of colonies on plates using imaging techniques has also been used (Dykes, 1999).

With data on τ available, it is then necessary to express the mean and variability of these values in a form which can be adapted to modelling. The most common approach is to fit different probability distributions to the data, although insufficient data have been collected thus far to allow any clear indication of the best distributions to be used. Several have been suggested, including Extreme Value (Smelt et al., 2002), Gamma (Métris et al., 2003), and Normal (Wu et al., 2000). This approach is complicated by the fact that exposure to stressors such as heat or starvation can result in longer and increasingly variable lag times (Dykes, 1999; Smelt et al., 2002).

The objective of the present study was to gather sufficient information on τ to determine the best distribution function to use for modelling. The work was carried out with a strain of *Escherichia coli* O157:H7 and several genetically modified constructs derived from it, with a view to using these strains to model expression of genes associated with stress and the stationary phase.

2. Materials and methods

2.1. Strains and culture conditions

E. coli O157:H7 (Ent C9490), isolated from the Jack-In-The-Box outbreak of undercooked meat, was used. Several genetically modified *E. coli* O157:H7 Ent C9490 strains, harboring p*uspA::gfp*$_{uv}$, p*grpE::gfp*$_{uv}$, and p*rpoS::gfp*$_{uv}$ gene fusions, were also used and examined for possible differences in growth characteristics. Negative control strains of *E. coli* O157:H7 containing two different promoterless::gfp$_{uv}$ plasmids were included. The construction of the plasmids containing the stress genes *uspA*, *grpE*, and *rpoS* and fused to the reporter *gfp* gene was described previously (Funabashi et al., 2002; Zhang and Griffiths, 2003). All six strains were provided by Dr. M. Griffiths, Canadian Research Institute for Food Safety, University of Guelph, Guelph, ON, Canada, N1G 2W1.

All cultures were resuscitated from 0.3 ml of stock culture frozen (−25 °C) in glycerol, which was inoculated into 10 ml tryptic soy broth (TSB; Difco Laboratories, Detroit, MI, USA). For plasmid-containing strains, TSB was supplemented with ampicillin sodium salt (Sigma-Aldrich, Oakville, ON, Canada) at either 50 μg ml^{-1}, for p*uspA::gfp*$_{uv}$, p*grpE::gfp*$_{uv}$, and their negative control, or with 100 μg ml^{-1} for p*rpoS::gfp*$_{uv}$ and its negative control. Cultures were grown at 37 °C with shaking at 200 rpm for 24 h. The cultures were transferred (0.01%) to 100 ml of fresh TSB broth in 500-ml flasks and incubated under the same conditions for 16 h to obtain stationary phase cells for experimentation.

Cultures were streaked for purity onto tryptic soy agar (TSA; Difco Laboratories) plates containing ampicillin, and serotyped using *E. coli* O Antiserum O157 (Difco Laboratories) to confirm identity.

2.2. Bioscreen growth analysis

Serial ten-fold and two-fold dilutions of each 16 h culture were prepared in fresh TSB, to obtain seven dilutions from approximately 10^8 to 10^1 cfu ml^{-1}, and eight dilutions from approximately 0.5×10^3 to 0.3 cfu ml^{-1}, respectively. Ten replicate samples from each of the ten lower dilutions were inoculated (350 μl volumes) into wells of a Bioscreen plate (Labsystems Corporation, Helsinki, Finland). Twenty replicate samples of the remaining 5 less-populated dilutions were placed into the wells of a second plate. The plates were loaded into the Bioscreen C Analyzer System (Labsystems Corporation) operating at an incubation temperature of 37 °C. Absorbance measurements were determined using a wide band filter, at 10 min intervals, after shaking at medium intensity for 10 s, and data capture was by the Biolink V5.26 software. Plates were incubated for 24 h. The first dilution of each sample was spread plated onto TSA, incubated at 37 °C overnight, and colonies were enumerated.

From the Bioscreen software, time-to-detection (time required for a 0.05 increase in absorbance from zero time (t_0); t_d) results from each well were obtained, and a mean value was calculated for each dilution. For each strain examined, inoculum density (ln cfu well^{-1}) was plotted against mean t_d using GraphPad Prism Version 4.03 for Windows (GraphPad Software, San Diego, CA, USA). From linear regression analysis of these plots, slopes were derived and specific growth rates (μ) were calculated from the formula (McKellar and Knight, 2000) $\mu = -1/\text{slope}$. Four replicate experiments were done with *E. coli* O157:H7 p*uspA::gfp*$_{uv}$ and three replicate experiments were done with each of the other five strains.

For fitting of distributions, data from 20-well replicate dilutions which had less than or equal to 50% of the wells showing growth were pooled (1–3 dilutions, depending on the trial). Four replicate experiments with *E. coli* O157:H7 p*uspA::gpf*$_{uv}$, two replicate experiments with *E. coli* O157:H7 promoterless*::gfp*$_{uv}$ for p*uspA::gfp*$_{uv}$ and p*grpE::gfp*$_{uv}$, and three replicate experiments with the remaining four of the six test strains (*E. coli* O157:H7 parent, p*grpE::gfp*$_{uv}$, p*rpoS::gpf*$_{uv}$, and promoterless*::gfp*$_{uv}$ for *rpoS*) were included in this data. To compare results obtained using a much larger sample size, seven additional experiments were carried out with 100 instead of 20 sample replicates. The two promoterless negative control *E. coli* O157:H7 strains were used in these trials; three replicate experiments were done with *E. coli* O157:H7 promoterless*::gfp*$_{uv}$ for p*uspA::gfp*$_{uv}$ and p*grpE::gfp*$_{uv}$; and four replicate experiments were done with *E. coli* O157:H7 promoterless*::gpf*$_{uv}$ for p*rpoS::gfp*$_{uv}$. For each experiment, two samples of dilute cultures, which were expected to contain single cells, were each dispensed into 100 wells of Bioscreen plates. The t_d values of replicate samples from dilutions with $\leq$50% of wells showing growth were examined as above.