Food Microbiology xxx (2014) 1–6

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Individual cell heterogeneity as variability source in population dynamics of microbial inactivation

Zafiro Aspridou, Konstantinos P. Koutsoumanis*

Laboratory of Food Microbiology and Hygiene, Department of Food Science and Technology, School of Agriculture, Forestry and Natural Environment, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece

Keywords:
Salmonella enterica
Microbial inactivation
Variability
Individual cell

A B S T R A C T

A statistical modeling approach was applied for describing and evaluating the individual cell heterogeneity as variability source in microbial inactivation. The inactivation data (N_t vs time) of *Salmonella enterica* serotype Agona, with initial concentration $N_0 = 10^9$ CFU/ml in acidified tryptone soy broth (pH 3.5), were transformed to $(N_0 - N_t)/N_0$ vs time leading to the cumulative probability distribution of the individual cell inactivation times (t_i), which was further fitted to a variety of continuous distributions using @Risk software. The best-fitted t_i distribution (Gamma) was used to predict the inactivation of *S.* Agona populations of various N_0 using Monte Carlo simulation, with the number of iterations in each simulation being equal to N_0 and the number of simulations representing the variability of the population inactivation behavior. The Monte Carlo simulation results for a population with $N_0 = 10{,}000$ CFU/ml showed that the variability in the predicted inactivation behavior is negligible for concentrations down to 100 cells. As the concentration decreases below 100 cells, however, the variability increases significantly. The results also indicated that the *D*-value used in deterministic first order kinetic models is valid only for large populations. For small populations, *D*-value shows a high variability, originating from individual cell heterogeneity, and, thus, can be better characterized by a probability distribution rather than a uniform value. Validation experiments with small populations confirmed the variability predicted by the statistical model. The use of the proposed approach to quantify the variability in the inactivation of mixed microbial populations, consisting of subpopulations with different probability distributions of t_i, was also demonstrated.

1. Introduction

Mathematical models have been traditionally used to predict microbial behavior in foods. With specific reference to microbial survival, significant progress has been made, since the classical study of Bigelow in 1921, in describing the inactivation kinetics of food-related microorganisms. An increased number of studies have reported deviations from log-linear death (Cerf, 1977) and attempted to incorporate them in mathematical models (Couvert et al., 2005; Fernández et al., 1999; Mafart et al., 2002; Peleg and Cole, 1998; van Boekel, 2002). As a result, a plethora of primary models have been proposed for the description of various shapes of inactivation curves deviating from first order kinetics, including both linear (with or without shoulder/tail) and sigmoidal curves.

Despite the progress in this area, the majority of microbial inactivation models are based on deterministic approaches without taking into account the variability of factors affecting microbial responses (Membré et al., 2006). In the context of a risk analysis framework, currently applied at the European and international level for food safety management, the importance of variability of biological and natural phenomena is widely recognized (FAO/WHO, 2008). Deterministic inactivation models that provide point estimates are generally not optimal to satisfactorily manage safety of foods (Augustin et al., 2010; Couvert et al., 2010; Koutsoumanis and Angelidis, 2007). Indeed, if, for instance, the consequences of unacceptable levels of a surviving pathogen in a food after processing are grave, the knowledge only of the mean population decline is unlikely to be a sufficient basis for processing design. On the other hand, the use of the 'worst-case' scenario approach in food processing leads to unrealistic estimations with negative impact on food quality.

The major sources of variability affecting microbial responses include: (i) the initial pre- and post-processing contamination, (ii) variability in processing factors, (iii) variability in the food characteristics (pH, a_w etc.), (iv) variability of storage conditions (chill chain), and (v) biological variability. An important component of

* Corresponding author. Tel./fax: +30 2310991647.
E-mail address: kkoutsou@agro.auth.gr (K.P. Koutsoumanis).

http://dx.doi.org/10.1016/j.fm.2014.04.008
0740-0020/

the latter source is the heterogeneity in the individual cell behavior. The variability of individual cell division and growth has received increasing attention in the recent years (Guillier and Augustin, 2006; Koutsoumanis, 2008; Koutsoumanis and Lianou, 2013; Métris et al., 2008; Pin and Baranyi, 2006; Smelt et al., 2008). Pin and Baranyi (2006) studied the division times of single cells and used the distributions of these division times to describe the variability in the growth of microbial populations through a birth model. Koutsoumanis and Lianou (2013) reported a highly heterogeneous behavior in the colonial growth of *Salmonella enterica* serotype Typhimurium individual cells. They developed a model that provides stochastic growth curves demonstrating that the growth of single cells or small microbial populations is a pool of events each one of which has its own probability to occur. In contrast to microbial growth, very limited information is available regarding the role of individual cell heterogeneity in microbial inactivation. Deterministic inactivation models do not take into account heterogeneity in the resistance of individual cells to a lethal stress (Casolari, 1988) which, as in the case of growth, can be a significant source of variability in microbial inactivation during food processing.

The objective of the present study was to evaluate and describe the individual cell heterogeneity as a source of variability in population dynamics of microbial inactivation. For this, a statistical modeling approach was applied providing predictions for the time to a certain decrease in the microbial population in the form of probability distribution. The proposed approach may contribute to the development of risk-based processing designs and the improvement of the accuracy of risk assessment models.

2. Materials and methods

2.1. Bacterial strain and inoculum preparation

The bacterial strain used in this study was a *S. enterica* serotype Agona animal isolate, kindly provided by Dr. Martin Wiedmann (Cornell University, Ithaca, New York). Stock culture of the strain was stored frozen (−70 °C) onto Microbank™ porous beads (Pro-Lab Diagnostics, Ontario, Canada), while working cultures were stored refrigerated (5 °C) on tryptone soy agar (TSA; Lab M Limited, Lancashire, United Kingdom) slants and were renewed bimonthly. The strain was activated by transferring a loopful from the TSA slants into 10 ml of tryptone soy broth without dextrose (TSB-G, Lab M Limited) and incubating at 37 °C for 18 h.

The 18-h culture of the tested strain was centrifuged (model PK 120R, Thermo Electron Corporation, Waltham, MA, United States) at 6000 rpm for 20 min at 4 °C. The harvested cells were washed with quarter strength Ringer's solution (Ringer's solution, Lab M Limited) and centrifuged as described previously. The harvested cells of the washed culture were resuspended in the acid challenge medium (see below) so that three different inoculum levels were attained: approximately 10^9, $10^{3.5}$ and $10^{2.5}$ CFU/ml. More specifically, for an inoculum concentration of approximately 10^9 CFU/ml, six 18-h cultures were mixed in pairs and were centrifuged, and the harvested cells from each of the three centrifugation tubes were washed with 1 ml of Ringer's solution, mixed and centrifuged again. For an inoculum concentration of approximately 10^3 and 10^2 CFU/ml, one 18-h culture was centrifuged, the harvested cells were resuspended in 9 ml of Ringer's solution, and a 2-ml aliquot of the proper serial decimal dilution was centrifuged again.

2.2. Inactivation trials

The acid challenge medium used was TSB-G acidified to a pH 3.5 with lactic acid (min. 78%, Sigma Aldrich, St. Louis, United States). The pH of the medium was adjusted to this value using a digital pH meter with an epoxy refillable pH probe (Orion 3-Star Benchtop; Thermo Electron Corporation, Beverly, MA, United States), and was also measured after autoclaving to assure that its value was not considerably altered by the sterilization process. Prior to inoculation, the acid challenge medium was pre-warmed at 25 °C, with this temperature being maintained throughout the trial. The total duration of the trials depended on the inoculum level used. Samples from bacterial suspensions were taken at regular time intervals in order to obtain effective kinetic analysis of microbial inactivation. Appropriate serial decimal dilutions of samples in Ringer's solution were surface plated on TSA. Surviving populations were determined after incubation of the plates at 37 °C for 72 h. Eight to ten independent trials were conducted for each inoculum size tested.

3. Results and discussion

The inactivation of *S.* Agona with initial concentration of 10^9 CFU/ml in acidified TSB-G (pH 3.5) is presented in Fig. 1. The data were initially fitted to a deterministic first order kinetic model:

$$N_t = N_0 * e^{-k*t} = N_0 * e^{-(\ln(10)/D)*t} \quad (1)$$

where: N_t (CFU/ml) and N_0 (CFU/ml) are the microbial populations at time t and 0, respectively, k (min^{-1}) is the inactivation rate, t (min) is time and D (min) is the D-value (time required for 1-log reduction). The estimated k from the above deterministic model was 0.104 min^{-1} and the D-value was 22.1 min.

Deterministic equations are the traditional means for modeling food processes and their effect on the lethality of microbial populations. Using a deterministic approach, the effect of a treatment is described with one single inactivation rate or D-value per particular condition, resulting in a single predicted value for the total microbial reduction or the time required for a certain decrease in the microbial population. In practice, however, most of the factors affecting microbial behavior can vary significantly. Among the various sources of variability in microbial responses, the heterogeneity in the individual cell behavior is of particular interest in the field of predictive microbiology. Most of the inactivation models in the literature have been developed based on data from large microbial populations (usually consisting of millions of cells) and describe the population as a whole, without considering the

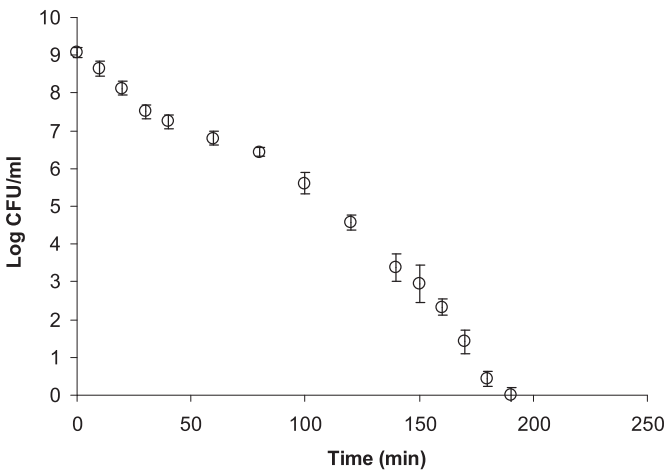

Fig. 1. Inactivation of *Salmonella enterica* ser. Agona with initial concentration of 10^9 CFU/ml in acidified tryptone soy broth (pH 3.5). Each point is the mean of 8–10 values. Error bars indicate the standard deviation values.

Please cite this article in press as: Aspridou, Z., Koutsoumanis, K.P., Individual cell heterogeneity as variability source in population dynamics of microbial inactivation, Food Microbiology (2014), http://dx.doi.org/10.1016/j.fm.2014.04.008