Non log-linear bacterial survival curves: a new primary model

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Introduction

Growth and thermal inactivation of microorganisms were extensively studied in order to get information on the microbiological impact of manufacturing process or formulation of foods. Nevertheless, there are still many unexplored points in the field of predictive microbiology: not controlled in order to be able to predict in the evolution of bacterial populations during the production or the storage of a food. In particular, slow decline of a population during storage, or during an acid or osmotic stress cannot be accurately predicted. This late development of the field of the non-thermal inactivation modelling is partly due to the heterogeneity of shapes of the bacterial survival curves which can be concave, convex, sigmoidal, or simply log-linear destruction. The same bacterial species can present the different shapes of curves according to experimental conditions(2, 6). As a consequence, a lot of primary models were published in literature. After having selected the primary model which yields the best fit of observed survival kinetics, authors deduce from it the $t_{4D}$ (the time of storage corresponding to a 4 log decline). This has the advantage of reflecting the evolution of the rate of inactivation as a function of the various investigated physicochemical factors regardless of the pattern of curves. On the other hand this modelling does not give information about the time for a given decimal log decline. This overall parameter simplifies the use of secondary model to predict the inactivation of microorganisms in unfavourable media. However, many models describing non log-linear curves of thermal inactivation were published (1, 3). The description of shapes relatively complex like sigmoids requires the implementation of new parameters which are generally without chemical, physics or biological meaning or which do not evolve in a logical way with the level of stress. Lastly, most of these models assume that the probability of survival tends towards an asymptote when time tends towards infinity. If their use does not set a problem for short processing times, they seem to over-estimate survival for prolonged times.

Materials and methods

Brain heart infusion broth was supplemented with HCl to give pH values ranging from 3.0 to 4.0. Overnight cultures of strains of Salmonella typhimurium (isolated from brine, ADRIA-France), or Listeria monocytogenes (isolated from pork-butchery, SOREDAB-France), were grown. Flasks of brain heart infusion were stored at 12°C under agitation (100 rpm) and sampled at appropriated intervals. The survivors were counted by plating on brain heart agar (automatic spiral plater, Don Whitley Scientific Limited, England). The fitting of the model to experimental data required the use of the nonlinear regression. The adjustments were done by the method of least squares. A nonlinear module of regression (lsqcurvefit, MATLAB 6.1, The Mathworks Inc, USA) was used.

Results and discussion

The observation of data from literature and of our own data led us to formulate precise requirements for the development of a new primary model which could suitably describe the kinetics of survival within the framework of non-thermal stress. The model should be able to describe a sigmoidal survival curves, but also to be able to fit with simple shapes. According to this model, the probability of survival of the bacterial population should tend towards zero when treatment time tends towards infinity. Moreover, the primary parameters should be stable and show a logical evolution with the level of stress, and according to the physiological initial state of the bacterial population. Lastly, the parameters should have a biological significance, chemical or biophysics, in order to facilitate their interpretation. The model of Whiting (6) satisfies these requirements. Derived from the logistic model, it is based on the
coexistence of two subpopulations which admit different resistances to the stress. This primary model, purely empirical, includes three parameters, each of which requires a secondary modelling. The replacement of the model of Whiting by a model with more mechanistic background bases and including one parameter less, would lead to an appreciable improvement.

The implementation of the model of Weibull to describe bacterial resistance to thermal stress was spread during last decades (4). In order to describe also sigmoidal kinetics of inactivation and by homology with work of Whiting (6), we assumed that the population is composed of two subgroups whose resistance to the stress follows a distribution of Weibull admitting the same value of the shape parameter. Regarding sigmoidal curves, this model presents a satisfying goodness of fit. Moreover, all parameters are easily interpretable. However, they are strongly correlated, that can lead to aberrant estimates, especially in cases of simplest shapes. In order to solve this problem, we have to make some simplifying assumptions. In our experiments, bacterial populations were in stationary phase. Resistance to stress is closely related to the initiation of mechanisms of resistances which appear with the entry in stationary phase (5). The subpopulations should correspond to the organisms having or not activated mechanisms of resistance to the stress. The model includes a parameter \( \alpha \) which corresponds to the decimal logarithms of the ratio of both initial subpopulations. This proportion is a function of subculture. The parameter \( p \) describes the form of the curve. Lastly, two resistance parameters characterise the intensity of the stress imposed on both subpopulations.

**Conclusion**

This paper presents a new model able to describe most patterns of non-thermal survival curves of the bacteria. It makes it possible to reflect the capacity of bacterial population to resist to the acid stress according to the physiological state of the bacterial population and to the intensity of the applied stresses. In the future, this model should make secondary modelling possible. Then, the influence of the physicochemical factors of stress, but also of physiological state of subculture on the rate of inactivation will be taken into account.

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