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A stochastic approach for integrating strain variability in modeling *Salmonella enterica* growth as a function of pH and water activity

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ABSTRACT

Strain variability of the growth behavior of foodborne pathogens has been acknowledged as an important issue in food safety management. A stochastic model providing predictions of the maximum specific growth rate (μ_{max}) of *Salmonella enterica* as a function of pH and water activity (a_w) and integrating intra-species variability data was developed. For this purpose, growth kinetic data of 60 *S. enterica* isolates, generated during monitoring of growth in tryptone soy broth of different pH (4.0–7.0) and a_w (0.964–0.992) values, were used. The effects of the environmental parameters on μ_{max} were modeled for each tested *S. enterica* strain using cardinal type and gamma concept models for pH and a_w, respectively. A multiplicative without interaction-type model, combining the models for pH and a_w, was used to describe the combined effect of these two environmental parameters on μ_{max}. The strain variability of the growth behavior of *S. enterica* was incorporated in the modeling procedure by using the cumulative probability distributions of the values of pH_{min}, pH_{opt} and a_{wmin} as inputs to the growth model. The cumulative probability distribution of the observed μ_{max} values corresponding to growth at pH 7.0–a_w 0.992 was introduced in the place of the model's parameter μ_{opt}. The introduction of the above distributions into the growth model resulted, using Monte Carlo simulation, in a stochastic model with its predictions being distributions of μ_{max} values characterizing the strain variability. The developed model was further validated using independent growth kinetic data (μ_{max} values) generated for the 60 strains of the pathogen at pH 5.0–a_w 0.977, and exhibited a satisfactory performance. The mean, standard deviation, and the 5th and 95th percentiles of the predicted μ_{max} distribution were 0.83, 0.08, and 0.69 and 0.96 h^{-1}, respectively, while the corresponding values of the observed distribution were 0.73, 0.09, and 0.61 and 0.85 h^{-1}. The stochastic modeling approach developed in this study can be useful in describing and integrating the strain variability of *S. enterica* growth kinetic behavior in quantitative microbiology and microbial risk assessment.

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1. Introduction

Quantitative microbiology, employing mathematical models that predict microbial behavior (growth or survival/inactivation), allows for the evaluation of microbial food-related risks, with the latter being critical for the development and implementation of effective mitigation strategies (Nauta, 2002). Due to the high level of variation characterizing microbial dynamics, the value of deterministic models (i.e., models that provide point estimates of microbial concentrations) in food safety management has been questioned (Nicolaï and Van Impe, 1996; Poschet et al., 2003). The information provided by deterministic models is often insufficient with regard to advanced quantitative microbiology applications, such as hazard analysis and critical control points (HACCP) and risk analysis projects (Poschet et al., 2003). Such a deficiency highlighted the need for the development of models capable of incorporating the variation of model parameters, and motivated the commencement of the so-called "stochastic predictive microbiology" (Nicolaï and Van Impe, 1996). Stochastic (or probabilistic) models take into account the variation of various factors affecting microbial behavior by using probability distributions of the input data, and provide predictions in the form of probability density functions instead of point estimates (Koutsoumanis et al., 2010; Poschet et al., 2003).

Accounting for the variation characterizing microbial growth, stochastic predictive models are expected to be more efficient than other traditional modeling approaches, by allowing for a balanced relationship between food safety management and cost-effectiveness of employed processes (Couvert et al., 2010; Juneja et al., 2003). Several stochastic predictive modeling approaches, aiming at quantifying and integrating different variation sources, have been described the last decade (Augustin et al., 2011; Delignette-Muller et al., 2006; Membré et al., 2005). The approaches embraced in the above and other studies account for variation on empirical data and/or model parameters, and are pertinent to various issues of food safety

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significance, such as quantitative microbial risk assessment (QMRA) and development of pathogen control interventions and food safety management systems.

The inherent differences in microbial behavior among identically treated strains of the same species constitute an important source of biological variability in microbiological studies (Whiting and Golden, 2002). As also commented by other investigators (Coleman et al., 2003; Delignette-Muller and Rosso, 2000; Pouillot and Lubran, 2011), the authors believe that intra-species variability of microbial growth may have a great impact on the "exposure assessment" component of QMRA, and, thus, should be systematically assessed and accounted for. Microbial growth variability among strains of a single bacterial species has been observed for several foodborne pathogens including *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica* and *Staphylococcus aureus* (Coleman et al., 2003; Dengremont and Membré, 1995; Fehlhaber and Krüger, 1998; Lianou et al., 2006). Nevertheless, only a limited number of microbiological studies have attempted to characterize the strain variability of the growth kinetic behavior of foodborne pathogens (Lianou and Koutsoumanis, 2011; Lindqvist, 2006; Nauta and Dufrenne, 1999; Whiting and Golden, 2002), while stochastic approaches explicitly taking into account this variability have been described mainly during the last decade (Couvert et al., 2010; Delignette-Muller and Rosso, 2000; Delignette-Muller et al., 2006; Koutsoumanis et al., 2010; Membré et al., 2005; Nauta and Dufrenne, 1999; Pouillot et al., 2003). Furthermore, in contrast to what is the case for other bacterial foodborne pathogens, the available research data on the variability of the growth behavior among strains of *S. enterica* are relatively few. The majority of relevant investigations have been conducted on a limited number of strains of the pathogen (Juneja et al., 2003; Membré et al., 2005; Oscar, 1998, 2000), and only a few studies have assessed growth kinetic differences among a large set of strains and under various environmental conditions (Fehlhaber and Krüger, 1998; Lianou and Koutsoumanis, 2011).

The objective of the present study was the development and validation of a stochastic model for integrating strain variability in modeling *S. enterica* growth. Beyond the scientific interest in quantifying the effect of growth environment on strain variability, a stochastic modeling approach such as the one developed in this study is expected to be useful in microbial risk assessment.

2. Materials and methods

2.1. Growth data of S. enterica strains

The growth kinetic data used in this work were maximum specific growth rate (μ_{max}) values corresponding to 60 *S. enterica* isolates, and were generated in a previous study undertaken in our laboratory (Lianou and Koutsoumanis, 2011). The tested strains were kindly provided by Dr. Martin Wiedmann (Cornell University, Ithaca, NY, USA), Dr. Constantin Genigeorgis (Aristotle University of Thessaloniki, Thessaloniki, Greece) and Dr. Daniil Sergelidis (Aristotle University of Thessaloniki), and were primarily isolates of human or animal (almost exclusively bovine) origin belonging to various serotypes: 18 of serotype Typhimurium, 10 of serotype Enteritidis, 9 of serotype Newport, 8 of serotype Heidelberg, and 15 of other serotypes (Lianou and Koutsoumanis, 2011). Briefly, in our previous study, aiming at characterizing the above *S. enterica* strains based on growth rates and evaluating the effect of growth conditions on the strain variability of the kinetic behavior of this organism, the growth kinetic behavior of the strains was assessed at 37 °C in culture broth of: (i) pH 7.0 and water activity (a_w) 0.992 (optimum growth conditions); (ii) pH 7.0 and a_w 0.983, 0.977 or 0.964; and (iii) pH 4.3, 4.5, 5.0 or 5.5 and a_w 0.992. The μ_{max} values corresponding to each strain and growth condition were estimated by means of absorbance detection times of serially decimally diluted cultures using an automated turbidimetric system. The detection times (h) of five serial decimal dilutions of the bacterial cultures were plotted against the natural logarithm of their initial concentrations, and μ_{max} values were determined by linear regression (Lianou and Koutsoumanis, 2011).

In order for the minimum growth requirements of the *S. enterica* strains to be better approached and the μ_{max} modeling as a function of pH to be facilitated, additional experiments assessing the growth kinetic behavior of the 60 strains of the pathogen were undertaken in the present study. More specifically, the growth behavior of the *S. enterica* strains was evaluated in tryptone soy broth (TSB; Lab M Limited, Lancashire, United Kingdom) of pH 4.0 (and 0.5% NaCl as part of its basal composition). The pH of TSB was adjusted to this value with HCl (min 37%; Sigma-Aldrich, Seelze, Germany) using a digital pH meter with an epoxy refillable pH probe (Orion 3-Star pH Benchtop; Thermo Electron Corporation, Beverly, MA, USA), and the growth experiments (one experiment with two samples per strain) were carried out following, overall, previously described procedures (Lianou and Koutsoumanis, 2011). However, given that the lowest pH value that could be evaluated in the context of the decimal dilution method exploited in our previous study was 4.3 (Lianou and Koutsoumanis, 2011), the growth kinetic behavior of the *S. enterica* strains at pH 4.0 was assessed using binal instead of decimal dilutions. More specifically, each 18-h culture of each one of the strains tested was decimally diluted in TSB of pH 4.0 to a concentration of approximately 10^7 CFU/ml, and then, 180-μl aliquots of five serial binal dilutions were added to 180 μl of TSB of pH 4.0 dispensed in 100-well microtiter plates. With the exception of the first dilution, the rest of the dilutions for each culture were attained by transferring 180-μl portions from one microtiter-plate well to the other, while 180 μl of the fifth dilution were discarded in order for all wells to contain the same culture volume (i.e., 180 μl). In this way, the range of initial bacterial concentrations obtained for each strain in the microtiter plates was approximately 10^6–10^4 CFU/well. Optical density measurements were taken at 30-min intervals using the automated turbidimetric system Bioscreen C (Oy Growth Curves Ab Ltd., Raisio, Finland) at an incubation temperature of 37 °C, with the rest of the experimental procedures as well as the estimation of the μ_{max} values being conducted as previously described (Lianou and Koutsoumanis, 2011).

2.2. Growth rate modeling

2.2.1. Growth models

The effect of pH on μ_{max} was modeled for each tested *S. enterica* strain using the cardinal type model of Rosso (Rosso et al., 1995):

$$\mu_{max} = \mu_{opt} \cdot \rho(\mathrm{pH})$$

$$\rho(\mathrm{pH}) = \begin{cases} 0, & \mathrm{pH} \le \mathrm{pH}_{min} \\ \dfrac{(\mathrm{pH}-\mathrm{pH}_{min}) \cdot (\mathrm{pH}-\mathrm{pH}_{max})}{(\mathrm{pH}-\mathrm{pH}_{min}) \cdot (\mathrm{pH}-\mathrm{pH}_{max}) - (\mathrm{pH}-\mathrm{pH}_{opt})^2}, & \mathrm{pH}_{min} < \mathrm{pH} < \mathrm{pH}_{max} \\ 0, & \mathrm{pH} \ge \mathrm{pH}_{max} \end{cases}$$

where pH_{min}, pH_{opt} and pH_{max} are the corresponding cardinal values, and μ_{opt} is the optimum value of the maximum specific growth rate (when $pH = pH_{opt}$). The effect of a_w on μ_{max} was modeled using the gamma concept and the model of Zwietering et al. (1996), with the gamma factor for a_w being slightly modified:

$$\mu_{max} = \mu_{opt} \cdot \gamma(a_w)$$

$$\gamma(a_w) = \left(\frac{a_w - a_{wmin}}{a_{w\,opt} - a_{wmin}}\right)^2$$

where a_{wmin} is the a_w value below which growth is not possible, and a_{wopt} is the a_w value at which the maximum specific growth rate is equal to its optimum value.

The values of pH_{min}, pH_{opt}, pH_{max} and a_{wmin}, were determined by fitting the estimated μ_{max} values for each tested strain to the above models

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