International Journal of Food Microbiology 149 (2011) 254–261

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

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

Alexandra Lianou, Konstantinos P. Koutsoumanis *

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

ARTICLE INFO

Article history:
Received 16 February 2011
Received in revised form 8 June 2011
Accepted 3 July 2011
Available online 13 July 2011

Keywords:
Salmonella enterica
Strain variability
Stochastic growth model

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.

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

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

0168-1605/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijfoodmicro.2011.07.001

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

using the Excel v4 format of the curve-fitting program TableCurve 2D (Systat Software Inc., San Jose, CA, USA). The a_{wopt} was set at 1 when the μ_{max} data were fitted to the gamma concept model.

As proposed by several authors (Buchanan et al., 1993; Wijtzes et al., 1993; Zwietering et al., 1992), discrete environmental conditions exert independent effects on microbial growth. Making this assumption, a multiplicative without interaction-type model (Augustin and Carlier, 2000; Panagou et al., 2003; Rosso et al., 1995), combining the above models for pH and a_w, was used to describe the combined effect of these two environmental parameters on μ_{max}:

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

where μ_{opt} is the maximum specific growth rate corresponding to optimum growth conditions.

2.2.2. Stochastic modeling approach

The intra-species 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 described above. The cumulative probability distribution of the estimated μ_{max} values corresponding to growth at pH 7.0–a_w 0.992 (regarded as optimum growth conditions) was introduced in the place of the model's parameter μ_{opt}. The introduction of the above probability distributions into the growth model was carried out using the custom cumulative function of the @RISK 4.5 for Excel software (Palisade Corporation, Newfield, NY, USA), and resulted in a stochastic model with its predictions, using Monte Carlo simulation (10,000 iterations), being distributions of μ_{max} values.

2.3. Model validation

The stochastic growth model developed in this study was evaluated against independent data which were not used for its development. In particular, the model was validated against growth kinetic data generated for the 60 strains of the pathogen at pH 5.0–a_w 0.977. The latter growth kinetic data were μ_{max} values corresponding to bacterial growth at 37 °C in TSB of the above characteristics, and were estimated as described previously (Lianou and Koutsoumanis, 2011). The pH of TSB was adjusted to 5.0 with HCl as previously practiced, while the a_w value of 0.977 was measured with an AquaLab water activity meter (Model series 3, Decagon Devices, Inc., Pullman, WA, USA) and corresponded to a NaCl (Merck, Darmstadt, Germany) concentration of 4.5% (w/v). The pH and a_w values of TSB were also measured after autoclaving to assure that they were not considerably altered by the sterilization process. The growth of the *S. enterica* strains at the above conditions was assessed in two independent experiments, with two samples per strain being analyzed at each experiment ($n=4$). Model validation was undertaken utilizing the Monte Carlo simulation technique with 10,000 iterations, performed with the @RISK 4.5 for Excel software. The validation of the stochastic approach was based on the comparative evaluation of the predicted and observed distributions of μ_{max}. The latter comparison was made graphically and quantitatively using the percent relative error (%RE) values (Delignette-Muller et al., 1995; Oscar, 2005a) for the mean, standard deviation and percentiles of the μ_{max} distributions based on the following equation:

$$\%\mathrm{RE} = \left[\left(x_o - x_p\right) / x_o\right] * 100$$

where x_o and x_p are the values of the above statistics for the observed and predicted distributions, respectively. The performance indices of bias factor (B_f) and accuracy factor (A_f) (Ross, 1996) were also determined for the purpose of evaluating the stochastic approach, using the following equations:

$$B_f = 10^{\left(\sum_{i=1}^{100} \log\left(Pi_{predicted} / Pi_{observed}\right) / 100\right)}$$

$$A_f = 10^{\left(\sum_{i=1}^{100} \left|\log\left(Pi_{predicted} / Pi_{observed}\right)\right| / 100\right)}$$

where $Pi_{predicted}$ and $Pi_{observed}$ are the values of the ith percentile of the predicted and observed generation time (GT) distributions, respectively.

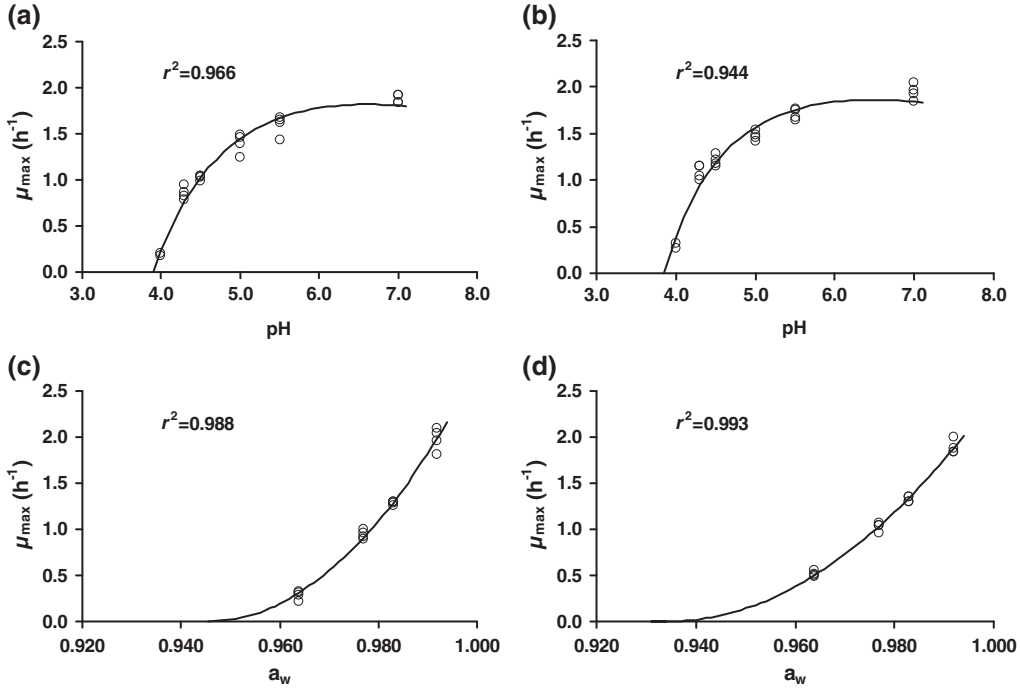

Fig. 1. Representative fittings (solid line) of the observed values (points) of maximum specific growth rate (μ_{max}), corresponding to four of the tested *Salmonella enterica* strains, to secondary models for pH (a, b) and water activity (a_w) (c, d), and the corresponding values of the coefficient of determination (r^2).

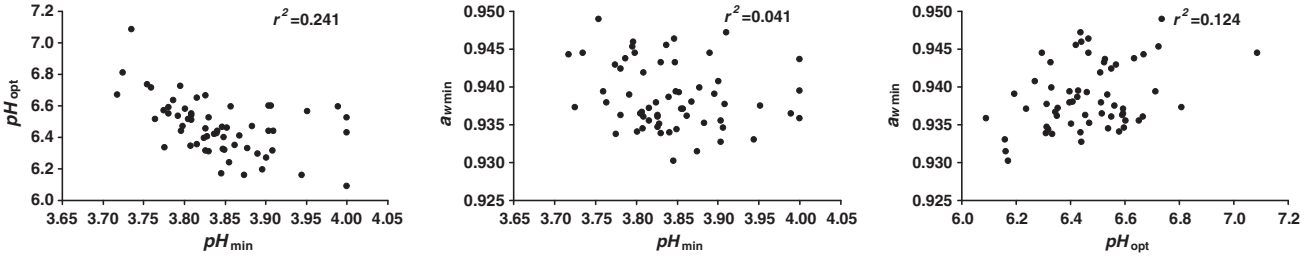

Fig. 2. Correlations of the estimated values of pH_{min}, pH_{opt} and a_{wmin} of 60 *Salmonella enterica* strains, and the corresponding values of the coefficient of determination of linear regression (r^2).

GTs were calculated from the corresponding values of μ_{max} using the equation $GT = \ln 2/\mu_{max}$.

3. Results and discussion

The growth kinetic data (i.e., μ_{max} values) exploited in this study were overall well-fitted to the secondary models used, with the mean (± standard deviation) coefficient of determination (r^2) for all the fitting trials ($n = 60$) being 0.934 (±0.034) and 0.983 (±0.009) for pH and a_w, respectively. Representative fittings of the observed μ_{max} values, corresponding to four of the tested *S. enterica* strains, to the secondary models used for pH and a_w are shown in Fig. 1. No considerable association was observed between the values of pH_{min}, pH_{opt} and a_{wmin} (Fig. 2), and similar findings have been also reported by other researchers for *L. monocytogenes* (Couvert et al., 2010; Delignette-Muller et al., 2006; Pouillot et al., 2003).

The mean and the 5th and 95th percentiles of the pH_{min} distribution were 3.84 and 3.75 and 3.99, respectively, while the corresponding values for the a_{wmin} distribution were 0.939 and 0.934 and 0.947. The mean and the 5th and 95th percentiles of the pH_{opt} distribution were 6.47 and 6.17 and 6.72, respectively. For pH_{max}, the mean and the 5th and 95th percentiles were 14.10 and 13.99 and 14.99, respectively. For the latter parameter, however, a high uncertainty was observed from the fitting of the μ_{max} data to the model due to the absence of data at superoptimal conditions. The estimated mean standard error of pH_{max} from the fitting was 5.2 while the corresponding value for pH_{min} was 0.08. Due to this high uncertainty, and given that the value of pH_{max} in the above range does not affect significantly the predictions of the model within its interpolation region (i.e., pH from 4.0 to 7.0), the distribution of pH_{max} was chosen not to be used in the stochastic model development; alternatively, pH_{max} was set at 14.0. Moreover, since the μ_{max} distribution corresponding to growth at a_w 0.992 was introduced in the place of the stochastic model's parameter μ_{opt}, and in order to avoid unrealistically high μ_{max} predictions, the a_{wopt} was set at 0.992 for simulation purposes.

The cumulative probability distributions of the values of pH_{min}, pH_{opt} and a_{wmin}, as well as of the parameter μ_{opt}, which were introduced into the growth model for the development of the stochastic modeling approach, are shown in Fig. 3. Via the introduction of the above distributions into the growth model and the use of Monte Carlo simulation, the model is converted into a stochastic one, which, by integrating strain variability, provides μ_{max} predictions in the form of distributions (Figs. 4 and 5). As demonstrated in Fig. 4, where the single effects of pH and a_w on the predicted μ_{max} are presented, the growth environment affected both the position and the shape of the predicted μ_{max} distributions. Furthermore, these stochastic predictions, obtained with simulations at various pH (assuming $a_w = a_{wopt}$) and a_w (assuming $pH = pH_{opt}$) values, were overall in good agreement with the observed μ_{max} values (i.e., dependent data), with most of the observed data being satisfactorily allocated within the predicted distributions (Fig. 4).

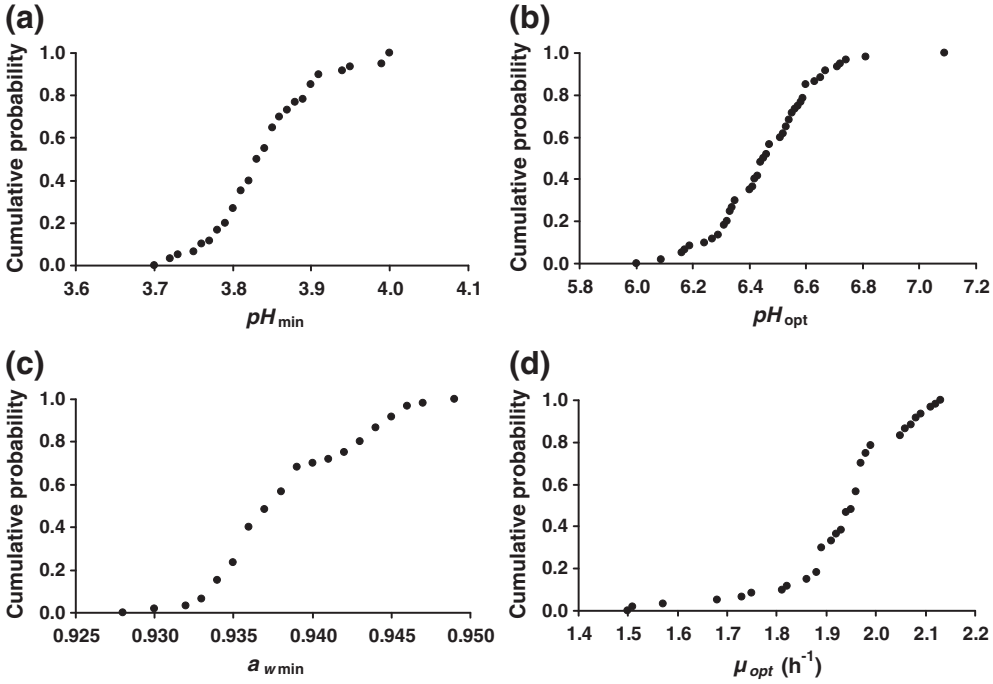

Fig. 3. Cumulative probability distributions of the values of pH_{min} (a), pH_{opt} (b) and a_{wmin} (c), and of the estimated maximum specific growth rate values corresponding to growth at pH 7.0–a_w 0.992 (μ_{opt}) (d) of 60 *Salmonella enterica* strains, introduced into the growth model for the development of a stochastic model integrating strain variability.

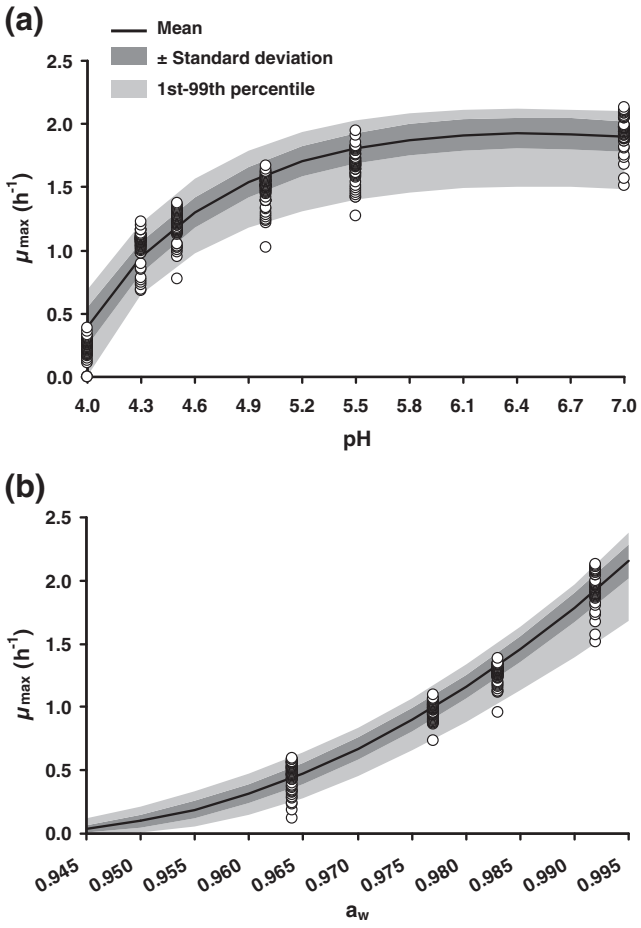

Fig. 4. Single effects of pH (assuming water activity $a_w = a_{wopt}$) (a) and a_w (assuming $pH = pH_{opt}$) (b) on the maximum specific growth rate (μ_{max}) of *Salmonella enterica*, as predicted by the developed growth model integrating strain variability and using Monte Carlo simulation (10,000 iterations). Points (○) represent the observed values of μ_{max}.

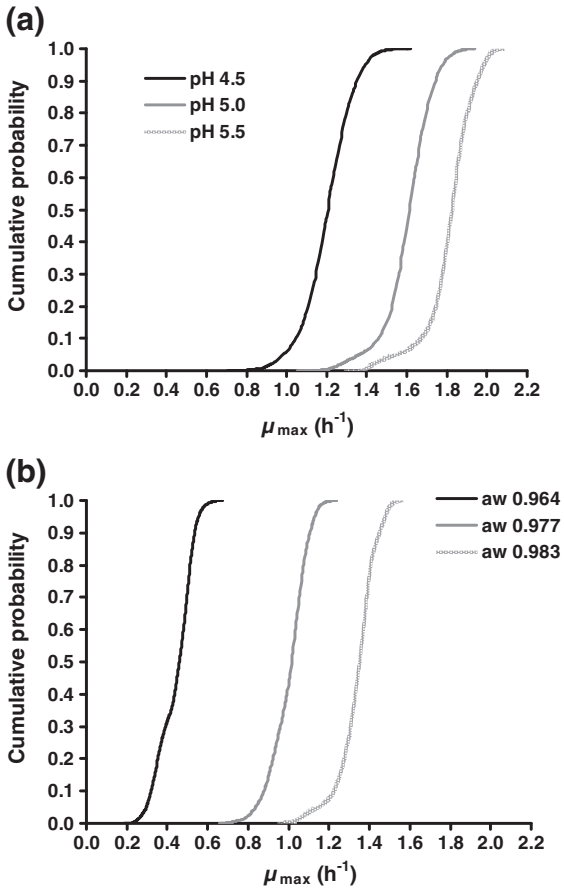

Fig. 5. Cumulative probability distributions of the maximum specific growth rate (μ_{max}) of *Salmonella enterica* at different pH (assuming water activity $a_w = a_{wopt}$) (a) and a_w (assuming $pH = pH_{opt}$) (b) values, as predicted by the developed growth model integrating strain variability and using Monte Carlo simulation (10,000 iterations).

The performance of the stochastic model was evaluated by comparing its predictions with independent data obtained at pH 5.0–a_w 0.977. As illustrated in Fig. 6, the predicted probability distribution of μ_{max} obtained with Monte Carlo simulation was fairly close to the probability distribution of the observed values. 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} (Table 1). With reference to deterministic models, the %RE and the B_f and A_f have been proposed for evaluating their performance during validation (Delignette-Muller et al., 1995; Oscar, 2005a; Ross, 1996). For evaluating the performance of the stochastic model developed in the present study,

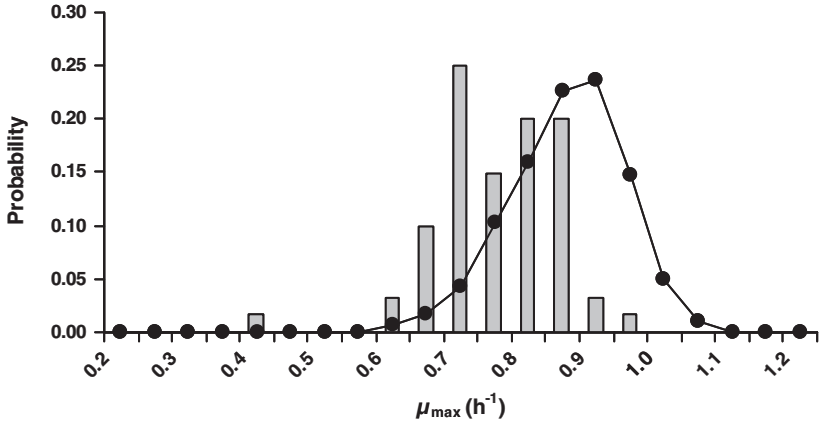

Fig. 6. Evaluation of the probability distribution of the values of maximum specific growth rate (μ_{max}) of *Salmonella enterica* predicted by the stochastic growth model (solid line), describing the combined effect of pH and water activity (a_w) on μ_{max}, against the probability distribution of independent data (i.e., μ_{max} values) corresponding to growth at pH 5.0–a_w 0.977 (bars).

Table 1
Validation of the stochastic growth model for *Salmonella enterica* against maximum specific growth rate (μ_{max}) data generated at pH 5.0 and water activity (a_w) 0.977, and using the percent relative error (%RE) values for the mean, standard deviation and percentiles of the μ_{max} distributions.

Statistics	μ_{max} (h^{-1})		
	Observed[a]	Predicted[b]	%RE[c]
Mean	0.73	0.83	−13.7
Standard deviation	0.09	0.08	11.1
1st percentile	0.50	0.61	−22.0
5th percentile	0.61	0.69	−13.1
10th percentile	0.63	0.72	−14.3
50th percentile	0.73	0.84	−15.1
90th percentile	0.83	0.93	−12.1
95th percentile	0.85	0.96	−12.9
99th percentile	0.90	1.00	−11.1

[a] Distribution of μ_{max} values obtained for 60 *S. enterica* strains during monitoring of growth in tryptone soy broth of pH 5.0–a_w 0.977.
[b] Distribution of μ_{max} values predicted by the stochastic model using Monte Carlo simulation (10,000 iterations).
[c] %RE = [(observed − predicted)/observed]*100.

the above indices were modified by replacing the observed and predicted values of the kinetic parameters with the respective distribution percentiles. The %RE values for the mean, standard deviation and percentiles of the μ_{max} distributions (Table 1) also indicate the satisfactory performance of the stochastic model. The mean (±standard deviation, $n = 100$) %RE of all the percentiles of the μ_{max} distributions was −13.8 (±2.1)%. It has been suggested that %RE values in the range of −30% (fail-safe) to 15% (fail-dangerous) delimit an "acceptable prediction zone" for model evaluation purposes (Oscar, 2005a; Ross et al., 2000). As demonstrated both by the %RE values for the μ_{max} distributions' percentiles and by Fig. 6, the prediction zone of the stochastic growth model described here falls within the acceptable one, with the model's predictions being exclusively localized in the fail-safe area. The B_f and A_f were estimated to be 0.88 and 1.14, respectively. When the B_f is used for model performance evaluations involving pathogens, the following interpretation has been proposed: (i) 0.90–1.05 can be considered as good; (ii) 0.70–0.90 or 1.06–1.15 can be considered acceptable; and (iii) <0.70 or >1.15 should be considered unacceptable (Mellefont et al., 2003). Based on the latter classification, the B_f estimated in the present study (i.e., 0.88) can be characterized as acceptable for model evaluation purposes. Although the above indices

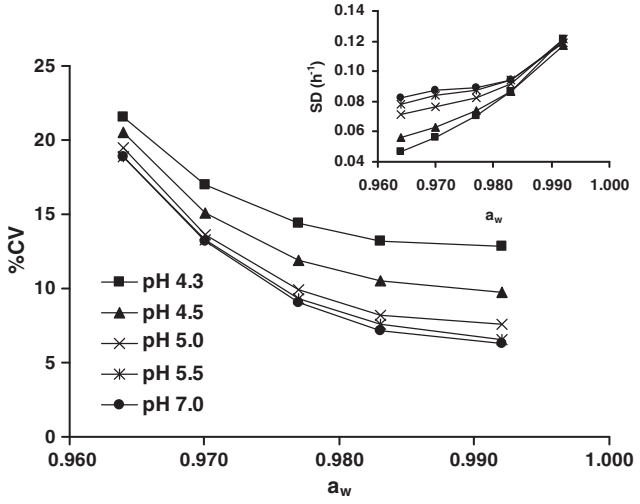

Fig. 7. Coefficient of variation (CV) and standard deviation (SD) of the maximum specific growth rate (μ_{max}) distributions of *Salmonella enterica*, obtained using the stochastic growth model and the Monte Carlo simulation technique (10,000 iterations), as a function of pH and water activity (a_w).

and their interpretation were not proposed for stochastic models, the information provided by their values as estimated in the present study could be regarded as an additional indication of the satisfactory performance of the developed stochastic modeling approach.

The impact of the growth environment on the strain variability of growth kinetics of foodborne pathogens has been indicated by various research data, with strain variability appearing to increase as growth conditions move away from the optimum for each microorganism (Begot et al., 1997; Fehlhaber and Krüger, 1998; Lianou et al., 2006). More specifically, the findings of our previous study, which was specifically designed to evaluate the effect of growth environment on the strain variability of the kinetic behavior of *S. enterica*, emphasized the need for the development of stochastic modeling approaches that take into account this effect (Lianou and Koutsoumanis, 2011). The growth model developed in this work, being based on research data referring to a sufficient number of strains and a wide range of growth conditions, incorporates such an effect, and is capable of providing predictions of the μ_{max} variability as a function of the environmental parameters studied (i.e., pH and a_w). Fig. 7 presents the evolution of the coefficient of variation (CV) of the μ_{max} distributions of *S. enterica*, obtained using the developed growth model and the Monte Carlo simulation technique (10,000 iterations), as a function of pH and a_w, along with the corresponding evolution of the standard deviation (SD). It is evident that, although the SD appears to decrease, the μ_{max} variability, as this is expressed by the CV of the predicted μ_{max} distributions, increases as the environmental conditions become more unfavorable for growth, both in terms of pH and a_w. Nevertheless, beyond this basic trend, additional observations can be made from this illustration. The predicted CV of μ_{max} appears to increase considerably (compared to optimum growth conditions) at pH values lower than 5.0 at a given a_w value, while important CV increases at a given pH value are observed at a_w values lower than 0.980. Furthermore, although both of the studied environmental parameters have individually the same net effect on μ_{max} variability (i.e., variability increases as pH or a_w become more unfavorable for growth), when considered in conjunction, the effect exerted by pH appears to be diminished as a_w values approach the growth/no growth boundary of the pathogen (Fig. 7).

When the stochasticity provided by a modeling approach, such as the one developed in this study, is embedded in a primary model, then more realistic predictions of microbial growth than those resulting from deterministic models are expected to be generated. For instance, incorporation of the developed stochastic model in the place of μ_{max} in a three-phase linear model (Buchanan et al., 1997), and assuming for all the tested *S. enterica* strains an initial population $N_0 = 2$ log CFU/ml and a physiological state $h_0 = 1$ (and, thus, a lag time $= 1/\mu_{max}$), resulted, using Monte Carlo simulation (10,000 iterations), in the stochastic growth prediction at pH 5.0–a_w 0.977 illustrated in Fig. 8; in this latter figure, the mean and the 1st and 99th percentiles of the predicted growth are presented in comparison with the observed growth curves (i.e., based on the observed μ_{max} values) of the 60 tested strains. It has been recommended that variability should be quantitatively expressed in risk estimates to the greatest scientifically achievable extent (Codex Alimentarius Commission, 2007). With the variability of the growth dynamics of foodborne pathogens being identified as one of the most important factors affecting the level of risk (Augustin et al., 2011; Pouillot and Lubran, 2011), its explicit consideration in QMRA approaches is expected to be of vital importance for their precision. Therefore, stochastic predictions of microbial growth such as the above (Fig. 8) are expected to be very useful in QMRA and particularly in exposure assessment.

Various sources of microbial growth variability have been addressed in stochastic modeling approaches including food characteristics, initial contamination level, individual cell behaviors, biological parameters such as cardinal values and growth parameters, storage conditions, and food microflora (Augustin et al., 2011; Couvert et al., 2010; Delignette-

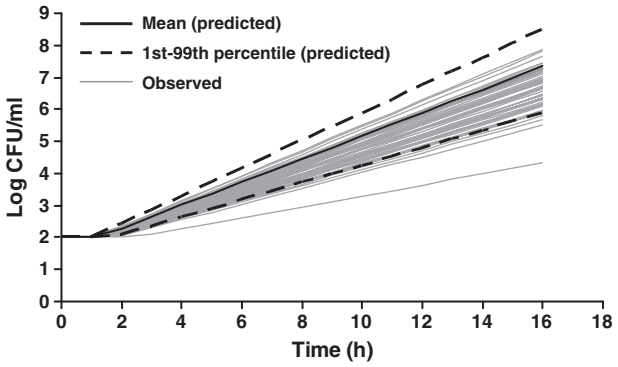

Fig. 8. Growth of *Salmonella enterica* at pH 5.0 and water activity 0.977, as predicted by a three-phase linear model (Buchanan et al., 1997). The growth rate (μ_{max}) variability among strains was integrated by introducing the developed stochastic model in the place of the primary model's parameter μ_{max}. Growth predictions were made assuming for all the tested *S. enterica* strains an initial population $N_0 = 2$ log CFU/ml and a physiological state $h_0 = 1$, and using Monte Carlo simulation (10,000 iterations). Growth predictions using the observed μ_{max} values for the 60 tested strains of the pathogen also were made (gray lines).

Muller et al., 2006). With regard to *S. enterica*, several models have been developed for predicting the growth of the organism as a function of various factors, such as temperature, pH, a_w (or NaCl concentration) and gel microstructure (Oscar, 2005b; Park et al., 2007; Theys et al., 2008), while stochastic modeling approaches have also been described (Oscar, 2002, 2008). Nevertheless, strain selection remains an important issue when it comes to the development of predictive models, and particularly when the latter are used for the purpose of exposure assessment (Nauta and Dufrenne, 1999). A worst-case situation, which is frequently embraced in predictive models, is a subjective situation and may introduce systematic biases into QMRA and result in risk overestimation, with the food industry being, most likely, unable to support the high cost associated with the implications of such a risk estimate (Begot et al., 1997; Nauta and Dufrenne, 1999). In addition, the use of mixtures of representative strains in model development for QMRA purposes also imposes a considerable risk of biased estimates (Juneja et al., 2003). Thus, an increasing interest in incorporating growth variability among strains of foodborne pathogens in predictive models has been observed the last decade.

With Monte Carlo analysis constituting a very useful tool for incorporating variation on experimental data in quantitative microbiology (Poschet et al., 2003), the vast majority of the modeling approaches implemented for the description and integration of strain variability are stochastic. And for this purpose, secondary models allowing for taking into account intra-species variability in their growth parameters are usually exploited (Couvert et al., 2010; Delignette-Muller and Rosso, 2000; Koutsoumanis et al., 2010; Pouillot et al., 2003). For instance, Delignette-Muller and Rosso (2000) described strain variability of *Bacillus cereus*, based on literature data, using probability distributions for the cardinal temperatures of the secondary model. Similarly, in a study undertaken recently by Couvert et al. (2010), and referring to the development of a stochastic modeling approach predicting *L. monocytogenes* distribution in foods throughout their shelf life, intra-species variability was described by fitting normal distributions to the cardinal values (temperature, pH and a_w) of 12 strains of the pathogen, as these were determined in laboratory media. In the risk assessment of *L. monocytogenes* in ready-to-eat foods, undertaken by the U.S. Food and Drug Administration and the U.S. Department of Agriculture Food Safety and Inspection Service (USFDA/USDA-FSIS, 2003), an approach known as "relative rate" approach was used to describe growth rate variability for inclusion in stochastic modeling (Ross and McMeekin, 2003). In the context of this approach, a relative rate relationship based on the square-root model (Ratkowsky et al., 1982) for temperature was used, with a probability distribution being assigned to the growth rate at a reference temperature (μ_{ref}) included in the secondary model, while T_{min} was assumed to be constant (Ross and McMeekin, 2003; USFDA/USDA-FSIS, 2003). A similar method was implemented in a study undertaken by Delignette-Muller et al. (2006), where, however, two sources of variability were taken into account: *L. monocytogenes* strain variability for T_{min} and product variability for μ_{ref}.

All the above approaches, attempting to characterize and incorporate strain variability in stochastic modeling, provide valuable information towards this direction, and definitely more realistic risk estimates than those neglecting this source of variability. However, the exclusively empirical character of some of the above methods and the limited number of strains evaluated in some others are parameters that may significantly compromise the validity of such approaches. Furthermore, another important factor that should be taken into account when developing such methods is the effect of the growth environment on the strain variability of microbial kinetic behavior. For instance, the "relative rate" approach, as described in the FDA/FSIS *L. monocytogenes* risk assessment, results in a variability which is not affected by the growth conditions. Nevertheless, as indicated by the data generated in our previous study (Lianou and Koutsoumanis, 2011) and confirmed by the stochastic model developed in this study (Fig. 7), this is not the case, and the effect of the growth environment on strain variability should be clearly and specifically expressed in modeling approaches accounting for this type of variability. Although assessment of the strain variability of lag time, in addition to that of μ_{max}, would be valuable, this variability was not investigated in our previous study (Lianou and Koutsoumanis, 2011) and, consequently, not integrated in the modeling approach developed in the present study. Estimation of this growth kinetic parameter (lag time) in the context of the applied methodology (i.e., optical density measurements) would require determination of bacterial concentration at the detection level for each growth curve (Dalgaard and Koutsoumanis, 2001), a task that, given the high number of the generated growth curves, would be almost unattainable in our study.

In order for QMRA to be credible, sufficient information on the distribution of the parameters implied in risk estimation is necessary, and both the uncertainty and variability of each parameter need to be taken into account (Nauta, 2000). Specifically with regard to the present study, some level of uncertainty is expected to originate from imprecise measurements inevitably encountered in the context of the experimental methods used, as well as from the fitting of the data to the models. Consequently, the growth rate variability predicted by the stochastic model described in this study is in fact a combination of the "true" strain variability and the above uncertainty. Ideally, uncertainty and variability should be separated in QMRA models, and such dissociation is generally made by using second-order Monte Carlo simulation (Delignette-Muller et al., 2006; Pouillot et al., 2003). Second-order modeling, however, requires a very high number of iterations and may become extremely time-consuming, and, thus, as an option is not attainable in the framework of a simple approach intended for the food industry.

In conclusion, the stochastic approach developed in the present study described adequately the μ_{max} variability among strains of *S. enterica* while modeling its growth as a function of pH and a_w. The developed model exhibited a satisfactory performance when validated against independent growth data acquired at a combination of the above two environmental parameters, with its predictions being exclusively localized in the fail-safe area and associated with acceptable prediction errors. The stochastic modeling approach presented here can be useful in describing and integrating the strain variability of *S. enterica* growth kinetic behavior in quantitative microbiology and microbial risk assessment. However, the developed model should be extended to also include the effect of temperature on the strain variability of *S. enterica* growth, and enhanced with appropriate validation studies in foods, if its contribution to the improvement of QMRA precision is to be assured.

Acknowledgments

This study has been carried out with the financial support of the Commission of the European Communities, Project ProSafeBeef "Food-CT-2006-36241". It does not necessarily reflect the Commission's views and in no way anticipates its future policy in this area.

References

Augustin, J.-C., Carlier, V., 2000. Mathematical modelling of the growth rate and lag time for *Listeria monocytogenes*. International Journal of Food Microbiology 56, 29–51.

Augustin, J.-C., Bergis, H., Midelet-Bourdin, G., Cornu, M., Couvert, O., Denis, C., Huchet, V., Lemonnier, S., Pinon, A., Vialette, M., Zuliani, V., Stahl, V., 2011. Design of challenge testing experiments to assess the variability of *Listeria monocytogenes* growth in foods. Food Microbiology 28, 746–754.

Begot, C., Lebert, I., Lebert, A., 1997. Variability of the response of 66 *Listeria monocytogenes* and *Listeria innocua* strains to different growth conditions. Food Microbiology 14, 403–412.

Buchanan, R.L., Smith, J.L., McColgan, C., Marmer, B.S., Golden, M., Dell, B., 1993. Response surface models for the effects of temperature, pH, sodium chloride, and sodium nitrite on the aerobic and anaerobic growth of *Staphylococcus aureus* 196E. Journal of Food Safety 13, 159–175.

Buchanan, R.L., Whiting, R.C., Damert, W.C., 1997. When is simple good enough: a comparison of the Gompertz, Baranyi, and three-phase linear models for fitting bacterial growth curves. Food Microbiology 14, 313–326.

Codex Alimentarius Commission, 2007. Working principles for risk analysis for food safety for application by governments. Available at http://www.codexalimentarius.net/web/publications.jsp2007.

Coleman, M.E., Tamplin, M.L., Phillips, J.G., Marmer, B.S., 2003. Influence of agitation, inoculum density, pH, and strain on the growth parameters of *Escherichia coli* O157:H7-relevance to risk assessment. International Journal of Food Microbiology 83, 147–160.

Couvert, O., Pinon, A., Bergis, H., Bourdichon, F., Carlin, F., Cornu, M., Denis, C., Gnanou Besse, N., Guillier, L., Jamet, E., Mettler, E., Stahl, V., Thuault, D., Zuliani, V., Augustin, J.-C., 2010. Validation of a stochastic modelling approach for *Listeria monocytogenes* growth in refrigerated foods. International Journal of Food Microbiology 144, 236–242.

Dalgaard, P., Koutsoumanis, K., 2001. Comparison of maximum specific growth rates and lag times estimated from absorbance and viable count data by different mathematical models. Journal of Microbiological Methods 43, 183–196.

Delignette-Muller, M.L., Rosso, L., 2000. Biological variability and exposure assessment. International Journal of Food Microbiology 58, 203–212.

Delignette-Muller, M.L., Rosso, L., Flandrois, J.P., 1995. Accuracy of microbial growth predictions with square root and polynomial models. International Journal of Food Microbiology 27, 139–146.

Delignette-Muller, M.L., Cornu, M., Pouillot, R., Denis, J.-B., 2006. Use of Bayesian modelling in risk assessment: application to growth *of Listeria monocytogenes* and food flora in cold-smoked salmon. International Journal of Food Microbiology 106, 195–208.

Dengremont, E., Membré, J.M., 1995. Statistical approach for comparison of the growth rates of five strains of *Staphylococcus aureus*. Applied and Environmental Microbiology 61, 4389–4395.

Fehlhaber, K., Krüger, G., 1998. The study of *Salmonella enteritidis* growth kinetics using Rapid Automated Bacterial Impedance Technique. Journal of Applied Microbiology 84, 945–949.

Juneja, V.K., Marks, H.M., Huang, L., 2003. Growth and heat resistance kinetic variation among various isolates of *Salmonella* and its application to risk assessment. Risk Analysis 23, 199–213.

Koutsoumanis, K., Pavlis, A., Nychas, G.-J.E., Xanthiakos, K., 2010. Probabilistic model for *Listeria monocytogenes* growth during distribution, retail storage, and domestic storage of pasteurized milk. Applied and Environmental Microbiology 76, 2181–2191.

Lianou, A., Koutsoumanis, K.P., 2011. Effect of the growth environment on the strain variability of *Salmonella enterica* kinetic behavior. Food Microbiology 28, 828–837.

Lianou, A., Stopforth, J.D., Yoon, Y., Wiedmann, M., Sofos, J.N., 2006. Growth and stress resistance variation in culture broth among *Listeria monocytogenes* strains of various serotypes and origins. Journal of Food Protection 69, 2640–2647.

Lindqvist, R., 2006. Estimation of *Staphylococcus aureus* growth parameters from turbidity data: characterization of strain variation and comparison of methods. Applied and Environmental Microbiology 72, 4862–4870.

Mellefont, L.A., McMeekin, T.A., Ross, T., 2003. Performance evaluation of a model describing the effects of temperature, water activity, pH and lactic acid concentration on the growth of *Escherichia coli*. International Journal of Food Microbiology 82, 45–58.

Membré, J.-M., Leporq, B., Vialette, M., Mettler, E., Perrier, L., Thuault, D., Zwietering, M., 2005. Temperature effect on bacterial growth rate: quantitative microbiology approach including cardinal values and variability estimates to perform growth simulations on/in food. International Journal of Food Microbiology 100, 179–186.

Nauta, M.J., 2000. Separation of uncertainty and variability in quantitative microbial risk assessment models. International Journal of Food Microbiology 57, 9–18.

Nauta, M.J., 2002. Modelling bacterial growth in quantitative microbiological risk assessment: is it possible? International Journal of Food Microbiology 73, 297–304.

Nauta, M.J., Dufrenne, J.B., 1999. Variability in growth characteristics of different *E. coli* O157:H7 isolates, and its implications for predictive microbiology. Quantitative Microbiology 1, 137–155.

Nicolaï, B.M., Van Impe, J.F., 1996. Predictive food microbiology: a probabilistic approach. Mathematics and Computers in Simulation 42, 287–292.

Oscar, T.P., 1998. Growth kinetics of *Salmonella* isolates in a laboratory medium as affected by isolate and holding temperature. Journal of Food Protection 61, 964–968.

Oscar, T.P., 2000. Variation of lag time and specific growth rate among 11 strains of *Salmonella* inoculated onto sterile ground chicken breast burgers and incubated at 25 °C. Journal of Food Safety 20, 225–236.

Oscar, T.P., 2002. Development and validation of a tertiary simulation model for predicting the potential growth of *Salmonella typhimurium* on cooked chicken. International Journal of Food Microbiology 76, 177–190.

Oscar, T.P., 2005a. Validation of lag time and growth rate models for *Salmonella* Typhimurium: acceptable prediction zone method. Journal of Food Science 70, M129–M137.

Oscar, T.P., 2005b. Development and validation of primary, secondary, and tertiary models for growth of *Salmonella* Typhimurium on sterile chicken. Journal of Food Protection 68, 2606–2613.

Oscar, T.P., 2008. Development and validation of a stochastic model for predicting the growth of *Salmonella* Typhimurium DT104 from a low initial density on chicken frankfurters with native microflora. Journal of Food Protection 71, 1135–1144.

Panagou, E.Z., Skandamis, P.N., Nychas, G.-J.E., 2003. Modelling the combined effect of temperature, pH and a_w on the growth rate of *Monascus rubber*, a heat-resistant fungus isolated from green table olives. Journal of Applied Microbiology 94, 146–156.

Park, S.-Y., Seo, K.-Y., Ha, S.-D., 2007. A response surface model based on absorbance data for the growth rates of *Salmonella enterica* serovar Typhimurium as a function of temperature, NaCl, and pH. Journal of Microbiology and Biotechnology 17, 644–649.

Poschet, F., Geeraerd, A.H., Scheerlinck, N., Nicolaï, B.M., Van Impe, J.F., 2003. Monte Carlo analysis as a tool to incorporate variation on experimental data in predictive microbiology. Food Microbiology 20, 285–295.

Pouillot, R., Lubran, M.B., 2011. Predictive microbiology models vs. modeling microbial growth within *Listeria monocytogenes* risk assessment: what parameters matter and why. Food Microbiology 28, 720–726.

Pouillot, R., Albert, I., Cornu, M., Denis, J.-B., 2003. Estimation of uncertainty and variability in bacterial growth using Bayesian inference. Application to *Listeria monocytogenes*. International Journal of Food Microbiology 81, 87–104.

Ratkowsky, D.A., Olley, J., McMeekin, T.A., Ball, A., 1982. Relationship between temperature and growth rate of bacterial cultures. Journal of Bacteriology 149, 1–5.

Ross, T., 1996. Indices for performance evaluation of predictive models in food microbiology. Journal of Applied Bacteriology 81, 501–508.

Ross, T., McMeekin, T.A., 2003. Modeling microbial growth within food safety risk assessments. Risk Analysis 23, 179–197.

Ross, T., Dalgaard, P., Tienungoon, S., 2000. Predictive modelling of the growth and survival of *Listeria* in fishery products. International Journal of Food Microbiology 62, 231–245.

Rosso, L., Lobry, J.R., Bajard, S., Flandrois, J.P., 1995. Convenient model to describe the combined effects of temperature and pH on microbial growth. Applied and Environmental Microbiology 61, 610–616.

Theys, T.E., Geeraerd, A.H., Verhulst, A., Poot, K., Van Bree, I., Devlieghere, F., Moldenaers, P., Wilson, D., Brocklehurst, T., Van Impe, J.F., 2008. Effect of pH, water activity and gel micro-structure, including oxygen profiles and rheological characterization, on the growth kinetics of *Salmonella* Typhimurium. International Journal of Food Microbiology 128, 67–77.

USFDA/USDA-FSIS (U.S. Food and Drug Administration/U.S. Department of Agriculture-Food Safety and Inspection Service), 2003. Quantitative assessment of the relative risk to pubic health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods. Available at http://www.fsis.usda.gov/Science/Risk_Assessments/index.asp2003.

Whiting, R.C., Golden, M.H., 2002. Variation among *Escherichia coli* O157:H7 strains relative to their growth, survival, thermal inactivation, and toxin production in broth. International Journal of Food Microbiology 75, 127–133.

Wijtzes, T., McClure, P.J., Zwietering, M.H., Roberts, T.A., 1993. Modelling bacterial growth of *Listeria monocytogenes* as a function of water activity, pH and temperature. International Journal of Food Microbiology 18, 139–149.

Zwietering, M.H., Wijtzes, T., de Wit, J.C., Van't Riet, K., 1992. A decision support system for prediction of the microbial spoilage in foods. Journal of Food Protection 55, 973–979.

Zwietering, M.H., de Wit, J.C., Notermans, S., 1996. Application of predictive microbiology to estimate the number of *Bacillus cereus* in pasteurised milk at the point of consumption. International Journal of Food Microbiology 30, 55–70.