A cardinal-type model with interaction to predict the growth and growth boundaries of *Salmonella* spp.

Le Marc Y.[1], Carduner M.L.[1], Skandamis P.[2], Nguyen Van Long N.[1]

[1]ADRIA Food Technology Institute, Quimper, France, [2]Laboratory of Food Quality Control and Hygiene, Department of Food Science and Human Nutrition, Agricultural University of Athens, Athens, Greece

INTRODUCTION AND OBJECTIVE

Salmonella is a leading cause of foodborne illness, and many types of food have been identified as sources of human cases of salmonellosis (including meat, produce and dairy products). It can cause serious illness, particularly in infants, older or frail adults, and individuals with weakened immune systems. The ability of salmonellae to adapt, survive or even thrive in a wide range of stressful environments (including the effects of acid and osmotic stress) been well documented and have prompted extensive microbiological modelling studies. However, to this date, there is no probabilistic model available that accounts for:

> factors such as temperature, pH, a_w, lactic acid,
> for strain variability on the growth rate and the growth limits of *Salmonella* spp. The objective of this study is to develop such a model and test it against well-characterized data in culture medium.

MATERIALS AND METHODS

A cardinal-type model (with an interaction term) was developed for the effects of temperature, pH, a_w, concentrations of undissociated lactic acid and the interactive effects of all these environmental factors on the growth rate of *Salmonella* spp.:

$$\mu_{max}^{Sal} = \mu_{opt,broth}^{Sal}\gamma(T)\gamma(pH)\gamma(a_w)\gamma\left([HLA]_{aq}\right)\xi \quad (1)$$

where μ_{max}^{Sal} is the maximum specific growth rate of *Salmonella*, $\mu_{opt,broth}$ is the μ_{max}-value at optimum conditions for growth in broth, $\gamma(T)$, $\gamma(pH)$, $\gamma(a_w)$ are the normalized effects (between 0 and 1) of temperature, pH and water activity, respectively and $\gamma([HLA]_{aq})$ is the normalized effect of undissociated lactic acid in the aqueous phase. The term ξ quantifies the effects of interactions between the environmental factors on μ_{max} (Le Marc et al., 2002, Augustin et al., 2005). The growth/no growth interface as predicted by Eq. (1) corresponds to the transition between conditions (ξ>0) and (ξ=0). The equations for the γ-terms and the "interaction term" ξ were derived from Le Marc et al. (2025).

The predictive model is defined by the following parameters: $\mu_{opt,broth}$, T_{min}, T_{opt} and T_{max} (minimum, optimum, maximum temperatures for growth, respectively), T_{mes} (the "Temperature of Maximum Enzyme Stability", introduced by Ratkowsky et al., 2005), pH_{min} (minimum pH for growth), aw_{min} (minimum water for growth) and MIC_{HLAC} (minimum inhibitory concentration of undissociated lactic acid in water). To account for strain variability, statistical distributions were used in the model instead of single values. For temperature and aw related parameters, the distributions were derived from μ_{max}-data sets generated in this study or obtained from the scientific literature. The distribution for pH_{min} was derived from values reported for different individual strains by Lianou and Koutsoumanis (2011). The distribution for MIC_{HLAC} was inferred from published MIC-values (Coroller et al., 2005) and using published logistic growth/no growth models for 4 different individual *Salmonella* strains (Paramithiotis et al., 2013). In the latter case, MIC_{HLAC} was set to the value of undissociated lactic acid associated with a predicted probability of growth of 0.1 (a level at which growth is considered as improbable) at pH 5.6 and 25°C.

The kinetics and growth boundaries of *Salmonella* in broth were predicted by means of 1D Monte Carlo simulations (10,000 iterations). The predictive ability of the model was assessed on data not used in the model development consisting of:

> μ_{max}-values collected from the core Combase FSA-IFR and UTAS datasets (www.combase.cc),
> growth/ no growth data from Combase and the scientific literature (1915 records for 7 different serovars, including 621 obtained in the presence of lactic acid).

RESULTS

PREDICTING THE GROWTH RATE OF SALMONELLA SPP.

Distributions for model parameters are indicated in Table 1. As in Couvert et al. (2010), normal distributions were used for the temperature, pH and a_w parameters. The individual values for MIC_{HLAC} did not show a Normal distribution pattern and as the number of strains was limited, a Beta-Pert distribution (commonly used when data is scarce and/or to model expert opinion) was used this parameter.

Parameter	Distribution	Number of strains	References for individual strain parameters
$\mu_{opt,broth}$ (h^{-1})	$\mathcal{N}(2.23, 0.18^2)$	7	This study
T_{min} (°C)	$\mathcal{N}(5.23, 0.86^2)$	10	This study, Oscar (2002,2003)
T_{opt} (°C)	$\mathcal{N}(39.58, 0.92^2)$	10	This study, Oscar (2002,2003)
T_{max} (°C)	$\mathcal{N}(46.16, 1.73^2)$	10	This study, Oscar (2002,2003)
T_{mes} (°C)	$\mathcal{N}(26.71, 1.00^2)$	10	This study, Oscar (2002,2003)
pH_{min}	$\mathcal{N}(3.84, 0.09^2)$	24	Lianou and Koutsoumanis (2011)
a_{wmin}	$\mathcal{N}(0.947, 0.009^2)$	6	This study
MIC_{HLAC} (mM)	*Pert* (5.1, 10.6, 14.8)	8	Coroller et al. (2005); Paramithiotis et al. (2013)

Table 1: *Distribution of model parameters used in the Monte Carlo simulations for predicting growth and growth/no growth of* Salmonella *spp.*

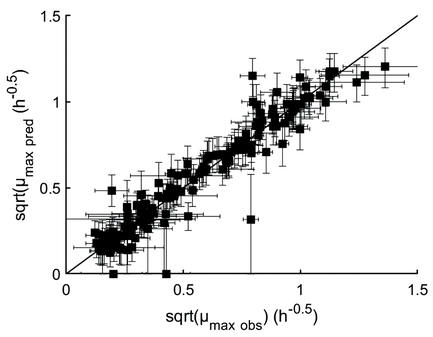


Figure 1: *Comparison between observed sqrt(μ_{max}) (ComBase core data in broth) and predicted values (Eq. (1)). The horizontal bars correspond to 95% confidence intervals of fitted μ_{max}-values (primary modelling) and the vertical bars to the 0.025 and 0.975 quantiles of the predicted distribution for μ_{max}.*

There is a good correspondence between the predicted and observed maximum growth rates from the ComBase core data with a 92% overlapping between the 95% confidence intervals on the estimated μ_{max}-values and the 95% limits of predictions (Fig. 1).

PREDICTING THE GROWTH/NO GROWTH OF *SALMONELLA* IN BROTH

The growth/no growth boundary predicted by the Monte Carlo simulation divides the environmental space in three areas (**A**: growth; **B**: growth possible for some strains only, **C**: no growth possible,) that are consistent with the experimental observations. The growth/no growth limits predicted by the interaction term were found to be consistent with experimental observations, as shown in Figs 2 to 5. The Negative Predictive Value (NPV, number of true negatives divided by the number of predicted "No Growth" conditions) is 99.3%.

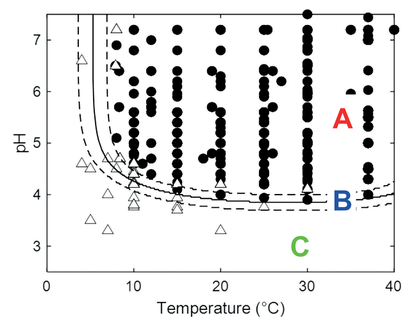


Figure 2: *Growth limits of* Salmonella *as a function of temperature and pH (a_w>0,99, no lactic acid). Comparison between predicted limits using Monte Carlo simulations (dotted lines: 0.025 and 0.975 quantiles, solid line: 0.5 quantile) and experimental observations (growth (●) and no growth (Δ)).*

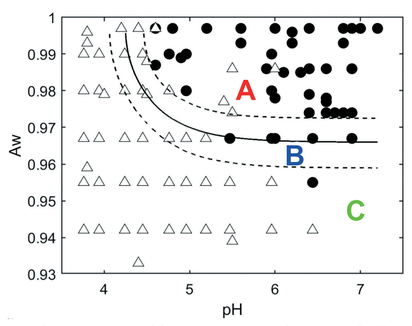


Figure 3: *Growth limits of* Salmonella *as a function of pH and a_w at 10°C (no lactic acid). Comparison between predicted limits using Monte Carlo simulations (dotted lines: 0.025 and 0.975 quantiles, solid line: 0.5 quantile) and experimental observations (growth (●) and no growth (Δ)).*

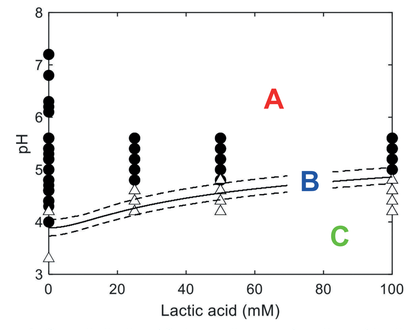


Figure 4: *Growth limits of* Salmonella *as a function of lactic acid and pH at 10°C and a_w>0,99. Comparison between predicted limits using Monte Carlo simulations (dotted lines: 0.025 and 0.975 quantiles, solid line: 0.5 quantile) and experimental observations (growth (●) and no growth (Δ)).*

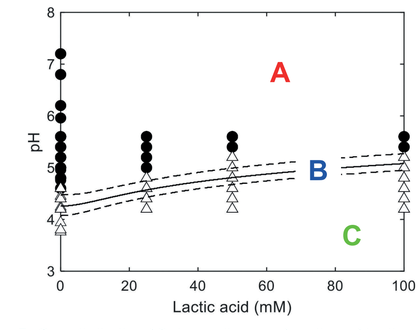


Figure 5: *Growth limits of* Salmonella *as a function of lactic acid and pH at 20°C and a_w>0,99. Comparison between predicted limits using Monte Carlo simulations (dotted lines: 0.025 and 0.975 quantiles, solid line: 0.5 quantile) and experimental observations (growth (●) and no growth (Δ)).*

CONCLUSIONS AND PERSPECTIVES

This probabilistic approach for growth/no growth modelling provides an accurate description of the growth limits of *Salmonella* spp. in broth. The model developed in this study can help the food industry to support and speed up development or reformulation of safe food recipes. Further work will focus on consolidating parameter distributions, combining the growth and growth/no growth model developped with thermal and non-thermal inactivation models in food.

References

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