Design of challenge testing experiments to assess the variability of microbial behaviors in foods

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Abstract

The assessment of the evolution of microorganisms naturally contaminating food must take into account the variability of biological factors, food characteristics and storage conditions. A research project involving eight French laboratories was conducted to quantify the variability of growth parameters of *Listeria monocytogenes* obtained by challenge testing in five foods. The residual variability corresponded to a coefficient of variation (CV) of approximately 20% for the growth rate (μ_{max}) and 120% for the parameter K (=μ_{max}.lag time). The between batches and between manufacturers variability was very dependent on the food tested and the CV of μ_{max} ranged from 0 to 80%. The initial physiological state variability led to a CV of 110% for the factor K. It appeared that repeating a limited number of challenge tests in different batches/manufacturers for different initial physiological states is often sufficient to assess the variability of the behavior of *L. monocytogenes* in a given food.

Keywords

Exposure assessment, biological variability, challenge testing, *Listeria monocytogenes*

Introduction

The assessment of the evolution of microorganisms that naturally contaminate food must take into account the variability of factors influencing the microbial behavior, i.e., biological factors, physico-chemical and microbial food characteristics, and storage conditions. The probabilistic software developed in the Sym'Previus project was designed to easily perform microbial exposure assessment and to combine the different source of variability with primary and secondary predictive microbiology models (Couvert et al., 2007). The biological variability of bacterial cardinal values is already set in the software but the variability of growth parameters, initial contamination, food characteristics and storage conditions must be specified by the users. It is really challenging to specify the variability of maximum growth rate and lag time of naturally contaminating microorganisms. The estimation of these parameters in natural conditions of contamination is generally impossible for pathogenic microorganisms and operators must usually perform challenge testing. A research project involving eight French laboratories was conducted to quantify the variability of growth parameters of *L. monocytogenes* obtained by challenge testing in five different foods. The objective was to evaluate the impact of within and between batches variability, between manufacturers variability, and microbial initial physiological state variability, on the

variability of the growth parameters to optimize the challenge testing methodology applied when evaluating the variability of the behavior of microorganisms in foods.

Materials and Methods

The following foods were studied: *i*) pâté from one batch, *ii*) smoked herring from four batches of two manufacturers, *iii*) cooked ham from seven batches and three manufacturers, *iv*) cooked chicken belonging to two batches of one manufacturer, and *v*) surimi salad from different batches of one manufacturer.

Each food was studied by two or three laboratories and was artificially contaminated with exponentially growing or starved cells of one strain of *L. monocytogenes* in order to evaluate the impact of physiological state on the growth parameters. Contaminated food samples were stored at 8°C and enumerations of *L. monocyotgenes* were performed on three samples at approximately 10 different times during the lag, the exponential and the stationary phases of the growth curve. Some experiments were replicated with the same batch of food, the same physiological state and the same laboratory to estimate the residual variability of growth parameters. pH and water activity (a_w) of foods were measured by laboratories to characterize the variability of physico-chemical characteristics of studied foods.

The maximum specific growth rate (μ_{max}) and the lag time (*lag*) were estimated for each growth curve by fitting the logistic with delay growth model (Pinon et al., 2004). In a second time, the variability of μ_{max} and of the product $K=\mu_{max}.lag$ representing the initial physiological state of contaminating cells was analyzed in order to determine the impact of the studied factors. Growth simulations were performed with the probabilistic software of Sym'Previus to combine the different variability sources in order to predict the growth curves of *L. monocyotgenes* or the probabilities to exceed given concentrations in foods.

Results and Discussion

The residual variability of μ_{max} was almost constant and a coefficient of variation (CV) of 20% was observed on average (Table 1). This variability was not explained by the variability of measured physico-chemical parameters since simulations performed for the species *L. monocytogenes* (12 strains) by only taking into account the observed variability of pH and aw of studied foods generated less variability for μ_{max} than the observed one (Table 1). This variability could then be linked to the variability of other not measured food characteristics or to the measurement uncertainty of μ_{max} when performing challenge testing. This result is not surprising since Baranyi and Roberts (1995) described repeatability standard errors of approximately 10% of the estimated growth rate in synthetic media. On the contrary, the residual variability of *K* was more pronounced and more variable with a mean CV of 120% (Table 1). This great variability of *K* can be easily explained by the difficulty for laboratories to experimentally reproduce specific bacterial physiological states. Since the residual variability of μ_{max} and *K* was relatively large, no significant effect of the laboratory performing the challenge test was observed for these two parameters.

The between batches and manufacturers effects on μ_{max} were very variables with CV ranging from 0 to 23% and from 0 to 81%, respectively (Table 1). The variability of *K* linked to the initial physiological state was relatively constant, which is consistent with the fact that only two physiological states were studied, and the CV was 110% on average.

The growth curves of *L. monocytogenes* generated for each food taking into account the biological variability, the variability of food characteristics and the variability of growth parameters summing the means of residual, between batches, between manufacturers and physiological state variances are shown in Figure 1. For pâté we observed that, the mean residual variability of *K* being larger than the observed one, the lag time was sometimes overestimated but the predicted behavior was relevant on the whole. For smoked herring, the mean between manufacturers variability lead to an overestimation of the observed variability while for the cooked ham, this mean variability was not sufficient to describe the observed one.

Table 1. Variability sources for growth parameters of *L. monocytogenes*.

				μ_{max}				K	
Food	pH (mean±SD)	a_w (mean±SD)	Variability (CV%)	Physico-chemical characteristics	Residual	Batch	Manu-facturer	Residual	Physio-logical state
Pâté	5.94±0.10	0.976±0.004	Input	_	16	ND*	ND	44	115
			Output	9	17	_	_	_	_
Smoked herring	6.37±0.08	0.966±0.009	Input	_	19	23	0	103	ND
			Output	26	32	40	40	_	_
Cooked ham	6.08±0.07	0.975±0.007	Input	_	20	0	81	135	103
			Output	15	25	25	75	_	_
Cooked chicken	6.30±0.19	0.974±0.008	Input	_	22	17	ND	141	88
			Output	17	23	29	_	_	_
Surimi salad	6.30±0.25	0.984±0.010	Input	_	21	0	ND	196	137
			Output	17	26	26	_	_	_
			Mean input		20	10	41	124	111

* ND not determined.

It seems thus that the residual variability of 20% for μ_{max} and 120% for K can be used to describe the variability of growth parameters for a given batch and a given physiological state but these parameters are too much varying for between batches and between manufacturers variability. Their impact on growth parameters is thus difficult to predict and several challenge tests are need. Furthermore it is hazardous to set the expected values of growth parameters with only one challenge test.

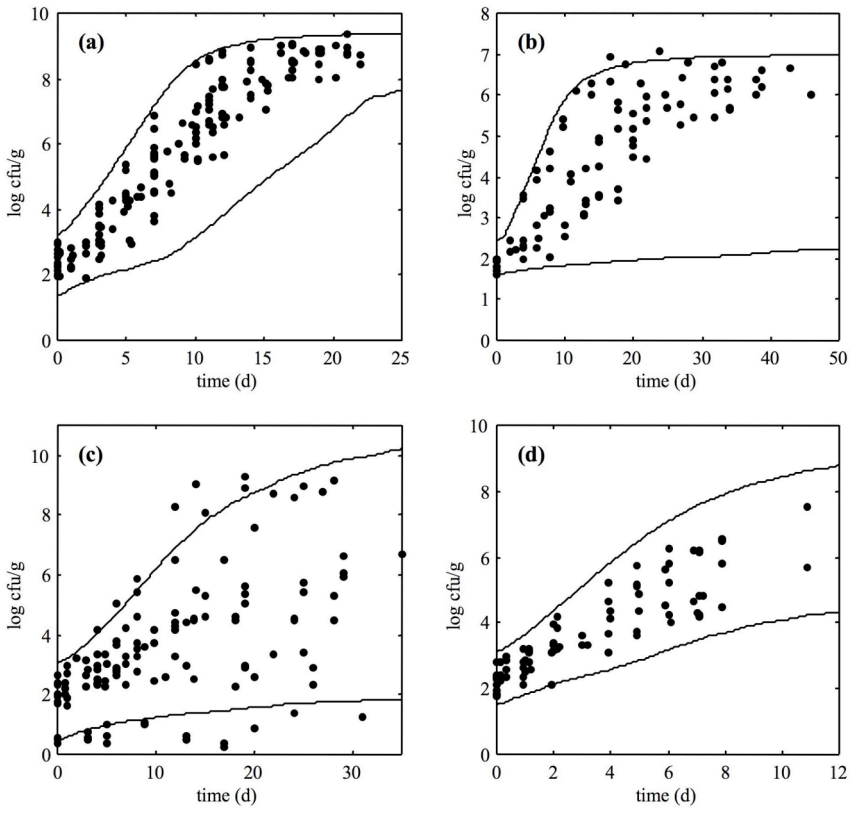

Figure 1. Observed (●) and simulated (95% confidence bands) growth of *L. monocytogenes* at 8°C in (a) pâté, (b) smoked herring, (c) cooked ham, and (d) surimi salad.

Then we proposed to perform three different challenge tests to estimate the expected values and standard deviations of μ_{max} and K. Depending on the studied factors influencing the growth, the challenge tests can be performed with three different batches or manufacturers and with three different physiological states. The Table 2 reports the results obtained when comparing this approach with the one consisting to use only one challenge test and fixing theoretical variances. The typical prediction errors (MARE) when predicting the probability to exceed a given concentration were lower when three challenge tests were performed and the dispersions of the relative errors (SDRE) were also lower.

Table 2. Mean absolute relative errors (MARE) and standard deviations of relative errors (SDRE) for predictions of probabilities P to exceed given concentrations of *L. monocytogenes* in foods stored at 8°C. The reference probabilities are those obtained by using all the challenge tests performed.

		1 kinetic		3 kinetics	
Food	Variability sources	MARE (%)	SDRE (%)	MARE (%)	SDRE (%)
Pâté (P>7 log cfu/g 8 days)	residual	99	135	60	71
Smoked herring (P>6 log cfu/g 15 days)	residual, batch, manufacturer	63	86	42	41
Cooked ham (P>6 log cfu/g 10 days)	residual, batch, manufacturer, physiological state	126	154	35	20
Cooked chicken (P>6 log cfu/g 8 days)	residual, batch, physiological state	55	67	24	14
Surimi salad (P>7 log cfu/g 10 days)	residual, batch, physiological state	37	43	4	–

Conclusion

The implementation of challenge tests to assess the variability of the growth parameters of foodborne pathogens is a keystone because the impact of the different sources of variability is unpredictable. By reproducing challenge tests in three different conditions it seems possible to satisfactorily evaluate the impact of between batches, between manufacturers and initial physiological state on growth parameters.

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References

Baranyi J. and Roberts T.A. (1995) Mathematics of predictive food microbiology. *International Journal of Food Microbiology* 26, 199-218.

Couvert O., Augustin J.-C., Buche P., Carlin F., Coroller L., Denis C., Jamet E., Mettler E., Pinon A., Postollec F., Stahl V., Zuliani1 V. and Thuault D. (2007) Optimising food process and formulation through Sym’Previus.. 5th International Conference Predictive Modelling in Foods, September 16-19, 2007, Athens, Greece.

Pinon A., Zwietering M., Perrier L., Membré J.-M., Leporq B., Mettler E., Thuault D., Coroller L., Stahl V. and Vialette M. (2004) Development and validation of experimental protocols for use of cardinal models for prediction of microorganism growth in food products. *Applied and Environmental Microbiology* 70, 1081–1087.