MATHEMATICAL MODELING OF *LACTOBACILLUS VIRIDESCENS* AND *LACTOBACILLUS SAKEI* GROWTH AT SIX DIFFERENT TEMPERATURES

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ABSTRACT – Lactic acid bacteria are the main microorganisms group responsible for the spoilage of refrigerated vacuum-packaged meat products, such as ham, sausage, chicken breast, among others. The main of this study was to model the growth of *Lactobacillus viridescens* and *Lactobacillus sakei* in culture medium at different temperatures. In this study, four primary growth models (Gompertz, Logistic, modified Logistic, and Baranyi-Roberts) were compared using the indices R^2, MSE, bias, and accuracy factor. The Gompertz model presented the best fit and was used for obtaining the growth parameters (lag phase duration (λ), maximum specific growth rate (μ), and microbial population increase (A)) for *L. viridescens* and *L. sakei* at six growth temperatures (4, 8, 12, 16, 20 and 30°C). From this study it can be seen that the growth of lactic acid bacteria is strongly influenced by the temperature of storage even under refrigerated conditions.

1. INTRODUCTION

Lactic acid bacteria group (LAB) was identified as the main spoilage population of vacuum-packaged and modified atmosphere meat products, and other processed products stored under refrigeration temperatures. The most frequent strains of LAB in meats and meat products are: *Lactobacillus sakei, Lactobacillus viridescens, Lactobacillus plantarum, Lactobacillus curvatus, Leuconostoc mesenteroides, Carnobacterium piscicola, Carnobacterium divergens* (Hugas, 1998). The spoilage caused by LAB is primarily due to the production of metabolites, which cause undesirable alterations in appearance, texture and flavor, producing unpleasant odors and flavors, along with the discoloring and slime production on the product's surface (Samelis et al., 2000).

Temperature seems to be the most important factor that influences food spoilage as well as safety. Hence, cold chain control is very important to avoid rapid and uncontrollable microbial growth, which reduce product shelf-life and may endanger public health (Nychas et al., 2008).

The continuous progress of science and technology of food preservation involves the development of new food microbiology tools. The need of assuring microbiological safety

and high quality foods has been stimulating the application of predictive microbiology tools. Predictive microbiology basically focuses on the development of mathematical models to describe the growth of pathogens and spoilage microorganisms in food, helping predicting food shelf-life (Nakashima et al., 2000; Brul et al., 2008).

The so-called primary growth models are those that describe the behavior of microorganisms during storage time (Whiting, 1995). They use three parameters in the characterization of bacterial growth curve, i.e., the lag phase duration (λ), the maximum specific growth rate (μ), and the microbial population increase (A). Primary models such as Gompertz, Logistic, modified Logistic, and Baranyi-Roberts models are often used for fitting microbial growth data (Buchanan et al., 1997; Erkmen and Alben, 2002; Corradini and Peleg, 2005; Pal et al., 2008).

The main of this study was to independently research *L. sakei* and *L. viridescens* growth at different temperatures (4, 8, 12, 16, 20 and 30°C), comparing the ability of the aforementioned primary models (Gompertz, Logistic, modified Logistic, and Baranyi-Roberts) to describe the growth curves.

2. MATERIAL AND METHODS

Lactobacillus sakei (ATCC 15521) and *Lactobacillus viridescens* (ATCC 12706) were grown in MRS (Man**,** Rogosa and Sharpe) - *Lactobacillus* broth (Acumedia).

2.1. Growth Conditions

The inoculums were grown for 18 h in MRS broth at 30°C. After this period, the inoculums were used to determine microbial growth curves at different temperatures: 4 and 8°C (refrigeration temperature), 12, 16 and 20°C (temperature abuse) and 30°C (optimum bacterial growth temperature). Experiments were carried out in 160 mL of MRS broth with 1% (v/v) of inoculum in 250 mL Erlenmeyer flasks. The initial pH was adjusted at 6. The flasks were maintained in an incubator (Dist) and the microbial growth evolution was determined until the stationary stage. For each LAB, at each tested temperature, four growth curves were generated: two duplicates at two different days (independent trials). The exception was the *L. sakei*, for which two curves were generated at 16°C, 20°C and 30°C.

Samples of 2 mL were collected aseptically in a laminar flow device (CFLV-09, Vecol) at predetermined intervals, depending on the temperature of growth. LAB growth was determined by absorbance (abs) at 600 nm wavelength in a spectrophotometer (1105, Bel Photonics). The pH was measured by a pHmeter (V620, Analion). The growth curves were followed until the stationary phase was achieved, and were obtained by plotting $\ln(abs/abs_0)$ *versus* time. The term abs represents the absorbance at time *t* and abs_0 the initial absorbance.

2.2. Modeling L. sakei and L. viridescens Growth at Various Temperatures

The primary models (Gompertz, Logistic, modified Logistic, and Baranyi-Roberts) were fit to LAB growth curves. Table 1 shows the equations representing each primary model. The growth curves were fitted using Matlab 7 software (MathWorks™, Natick, MA, USA) with Gompertz, Logistic and modified Logistic models, and DMfit program - Baranyi and Roberts (1994) - (Excel add-in) to Baranyi-Roberts model.

Table 1. Primary growth models used to fit the growth of LAB

Primary models	**Equations**[a]
Gompertz	$\ln(abs/abs_0) = A.\exp\{-\exp[-B.(t-M)]\}$ $\mu = \frac{A.B}{e} \qquad \lambda = M - \frac{1}{B}$
Logistic	$\ln(abs/abs_0) = \frac{A}{[1+\exp[-B.(t-M)]}$ $\mu = \frac{A.B}{4} \qquad \lambda = \frac{(M-2)}{B}$
Modified Logistic	$\ln\left(abs/abs_0\right) = \frac{A}{(1+\exp[-B.(t-M)} - \frac{A}{(1+\exp(M.B))}$ $\mu = \frac{A.B}{4} \qquad \lambda = \frac{(M-2)}{B}$
Baranyi-Roberts	$abs = abs_0 + \mu.F(t) - \frac{1}{m}\ln\left(1+\frac{\exp(m.\mu.F(t))-1}{\exp(m(abs_{max}-abs_0))}\right)$ $F(t) = t + \frac{1}{\mu}\ln\left(\exp(-\mu.t)+\exp(-\mu.\lambda)-\exp(-\mu(t+\lambda))\right)$

[a] ln (abs/abs$_0$): is the logarithm of the cell density at time *t*; abs: absorbance at time *t*; abs$_0$: initial absorbance; A is the logarithmic microbial population increase; μ: maximum specific growth rate (h^{-1}); λ: lag phase duration (h); B is the relative growth rate at time M (h^{-1}), and M is the time required to reach the maximum rate growth (h); abs$_{max}$: maximum absorbance, m is the curvature parameter; e = 2.7182.

Mathematical models comparisons were performed using the statistical indices: correlation coefficient (R^2), mean square error (MSE), bias factor, and factor accuracy (Sutherland et al., 1994; Ross, 1996). These statistical indices are shown in Table 2.

Table 2. Statistical indices for comparison of the models

Statistical indices	Equation[a]
MSE	$MSE = \frac{\sum \left(Value_{observed} - Value_{predicted}\right)^2}{n - p}$
Bias factor	$bias\ factor = 10^{\left(\sum \frac{\log\left(Value_{predicted}/Value_{observed}\right)}{n}\right)}$
Accuracy factor	$accuracy\ factor = 10^{\left(\sum \frac{\left\|\log\left(Value_{predicted}/Value_{observed}\right)\right\|}{n}\right)}$

[a] n: number of experimental data; p: number of model parameters.

3. RESULTS AND DISCUSSION

Table 3 show the range of statistical indices values obtained for primary models for the six growth temperatures for *L. viridescens*. The range represents the lowest and the highest indices values obtained by fitting the models to the four replicate growth curves, at each investigated temperature. The calculated statistical indices showed that all models represented well the growth behavior of these LAB. However, visual analysis of these fittings (data not presented) showed that the Logistic and Baranyi-Roberts models did not describe well the lag phase of growth curves for various investigated situations. The same behavior was observed for *L. sakei*.

As it can also be observed in Table 3, statistical indices of the Logistic and Baranyi-Roberts models are slightly lower than the values observed for Gompertz and modified Logistic models, with a slightly superior performance for the Gompertz model, which was the only one that showed correlation coefficients above 0.99, for all temperatures. The lowest values of the mean square error were observed for the Gompertz model. Bias and accuracy factors closer to 1 were also obtained for the Gompertz model.

Through analysis of the statistical indices, the primary model that showed the best fit to growth curves of *L. viridescens* and *L. sakei*, at all investigated temperatures, was the Gompertz model. Therefore, this model was chosen for the calculation of the growth parameters of LAB. These results match those reported by Slongo et al. (2009) who studied the influence of pressure level and holding time during high pressure treatment of ham on LAB growth in vacuum-packaged sliced ham. These authors reported that both modified Gompertz and Logistic models were able to describe microbial growth in ham. However, Gompertz model had a slightly superior performance. Zwietering et al. (1990) compared several sigmoidal functions (Logistic, Gompertz, Richards, Schnute, and Stannard models) for describing *L. plantarum* growth curves in MRS broth at different temperatures (6°C to

42.8°C). These authors concluded that all growth curves were better fitted with the Gompertz model.

Table 3. Range of statistical indices values for Gompertz, Logistic, modified Logistic, and Baranyi-Roberts models, obtained by fitting the growth curves of *L. viridescens*

Temperature/ Indices	**Models**			
	Gompertz	**Logistic**	**Modified Logistic**	**Baranyi**
30°C				
R^2	0.998-0.999	0.995-0.999	0.996-0.999	0.992-0.998
MSE	0.003-0.004	0.002-0.009	0.001-0.007	0.004-0.012
Bias	1.002-1.015	0.985-0.993	0.990-0.998	0.945-1.033
Accuracy	1.039-1.047	1.034-1.045	1.026-1.065	1.063-1.100
20°C				
R^2	0.998-0.999	0.994-0.999	0.996-0.999	0.989-0.997
MSE	0.002-0.004	0.003-0.015	0.002-0.011	0.004-0.023
Bias	0.999-1.012	0.981-0.992	0.988-0.998	0.949-1.041
Accuracy	1.039-1.052	1.042-1.113	1.036-1.088	1.075-1.098
16°C				
R^2	0.994-0.998	0.997-0.999	0.997-0.999	0.994-0.997
MSE	0.003-0.010	0.001-0.005	0.001-0.005	0.003-0.007
Bias	0.997-1.027	0.982-1.005	0.985-1.013	0.966-1.003
Accuracy	1.042-1.075	1.030-1.051	1.022-1.055	1.033-1.091
12°C				
R^2	0.994-0.999	0.986-0.997	0.989-0.998	0.976-0.996
MSE	0.001-0.010	0.003-0.022	0.002-0.016	0.005-0.029
Bias	0.993-1.004	0.981-0.988	0.987-0.994	0.985-1.037
Accuracy	1.022-1.076	1.049-1.127	1.036-1.095	1.081-1.228
8°C				
R^2	0.998-0.999	0.994-0.998	0.996-0.998	0.991-0.996
MSE	0.001-0.002	0.003-0.006	0.002-0.004	0.004-0.008
Bias	0.999-1.005	0.986-0.992	0.991-0.997	1.004-1.066
Accuracy	1.024-1.035	1.041-1.062	1.032-1.052	1.033-1.111
4°C				
R^2	0.995-0.997	0.992-0.998	0.996-0.998	0.990-0.998
MSE	0.001-0.002	0.001-0.003	0.001-0.003	0.001-0.003
Bias	1.000-1.007	0.992-0.997	0.997-1.003	0.964-1.010
Accuracy	1.029-1.045	1.023-1.044	1.018-1.044	1.077-1.160

Figures 1 and 2 shows the growth curves of *L. viridescens* and *L. sakei*, respectively, at six different temperatures, fitted by the Gompertz model. Each temperature is exemplified by only one curve in each cultivation condition. It can be observed that the model describes well all the growth curves. Analyzing these figures, it is possible to verify the behavior of the two LAB in each storage condition, and that the temperature decrease causes the increase of the lag phase, and the decrease of the maximum specific growth rate and of the maximum population. The two strains had a very similar behavior. They just showed little difference on

the curves at 20°C and 16°C of *L. viridescens*, for which the growth curves were very similar, a behavior not observed for the other strain.

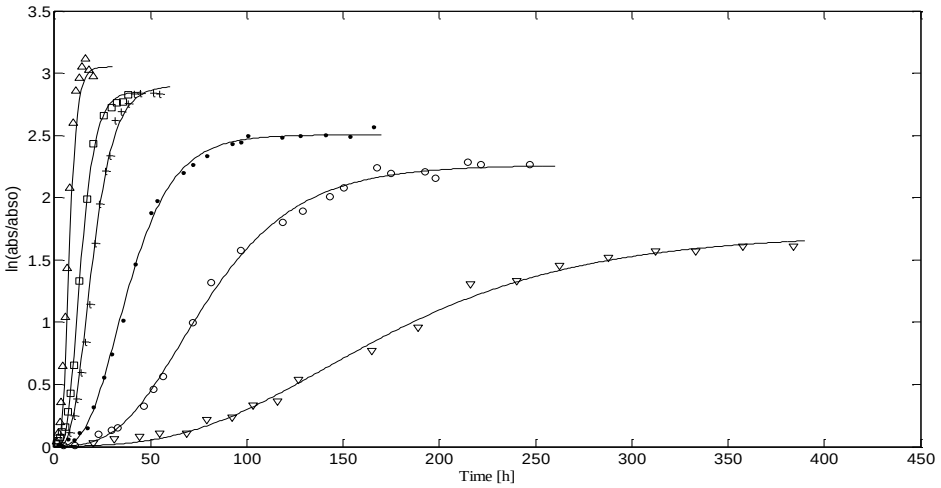

Figure 1. Example of *L. viridescens* growth curves in MRS broth at different temperatures. The lines represent the Gompertz model fit to experimental data (symbols). (∇) 4°C, (○) 8°C, (●) 12°C, (+) 16°C, (□) 20°C and (Δ) 30°C.

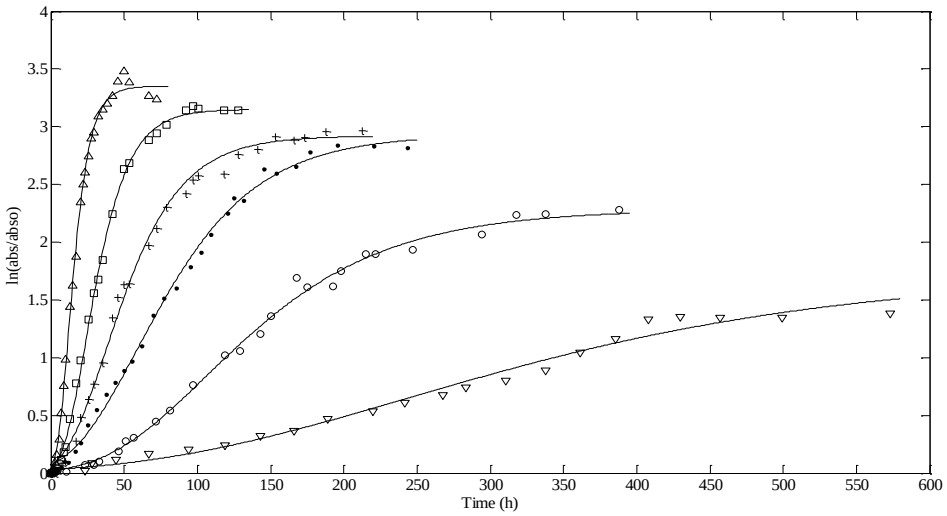

Figure 2. Example of *L. sakei* growth curves in MRS broth at different temperatures. The lines represent the Gompertz model fit to experimental data (symbols). (∇) 4°C, (○) 8°C, (●) 12°C, (+) 16°C, (□) 20°C and (Δ) 30°C.

In real storage and commercialization of foodstuffs, it is known that temperature fluctuations can occur. The results showed that small increases in temperature (variation of

4°C) interfered in all growth parameters. This way, it emphasizes the importance of controlling the chill chain, especially for refrigerated meat products that have a relatively short shelf-life, compared with other food products.

4. CONCLUSIONS

The primary model that showed the best fit to growth curves of *L. viridescens* and *L. sakei* at temperatures ranging from 4°C to 30°C was the Gompertz model. It is possible to conclude that the growth of LAB is strongly influenced by storage temperature, even under refrigeration conditions.

The shelf-life of a food product can be drastically reduced by temperature fluctuations and by temperature abuse, i.e., use of storage temperatures above the recommended for a given foodstuff. Although the experiments were developed in culture media, these datas could be helpful in the food industry, allowing for a better understanding of the behavior of the strains in meat products.

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