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Primary and secondary modeling of *Brochothrix thermosphacta* growth under different temperature and ph values

Leticia Dias dos Anjos GONÇALVES[1]*, Roberta Hilsdorf PICCOLI[2], Alexandre de Paula PERES[2], André Vital SAÚDE[3]

Abstract

Bochothrix thermosphacta is an optional aerobic psychrotrophic related to the meat deterioration and consequently loss of a refrigerated cargo contaminated. Predictive microbiology can be used as a quality assurance tool, since it allows the prediction of microbial response based in pass observations. This work aimed model the growth of *B. thermosphacta* under variation of pH and temperature. For this purpose, the experimental growth data were fitted to the primary models of Baranyi and Roberts and the modified Gompertz model and the data for the maximum rate of growth (μmax) were adjusted to Ratkowsky extended secondary model. The results showed us that the influence of temperature on growth parameters was more evident than pH. Since, by fixing the temperature the change of pH little altered the μmax. However, as the temperature rises the elevation at the μmax is considerable, for example the comparison of predicted values for μmax, when the temperature exceeds 4 °C to 12 °C, it is clear that these rates are more than double. Finally, it is emphasized that all tested models feature good fit to the experimental data, which makes them validated for prediction growth of *B. thermosphacta* in the same conditions tested experimentally.

Keywords: deterioration; refrigeration; predictive models.

Pratical Application: To predict the growth of *B. thermosphacta* under varying temperature and pH conditions.

1 Introduction

Generally, bacteria are absent or present in very low levels in muscle tissues from healthy animals. This is due to the inherent protective barriers (skins, leather) and the natural mechanisms of antimicrobial defense (lysozyme, antimicrobial peptides) of the living animal, which are destroyed at slaughter, so that the resulting meat become exposed to increasing levels of contaminants (Nychas et al., 2008). This fact leads in decreased in shelf- life of the meat, which is dependent on the number and type of contamination present initially and during storage conditions, especially temperature, pH, gas atmosphere (Russo et al., 2006).

With regard to pH, foods of low acidity (pH> 4.5), such as meat whose pH can range from 5.5 to 7.0 (Cárdenas et al., 2008), are more subject to microbial multiplication, both of pathogenic and deterioration species (Franco & Landgraf, 2005). An example of the effect of pH on meat microbial kinetics can be seen with work by Koutsoumanis et al. (2006), were able to observe the significant effect of ground meat pH (5.34 and 6.13) on a growth kinetics of *Pseudomonas*, *B. thermosphacta* and bacteria of the family *Enterobacteriaceae,* was observed, for example, for *B. thermosphacta* at 10 °C the growth rate doubles as the pH goes from 5.4 to 6.1.

Various conservation methods can be used to increase the shelf life of fresh meat, including refrigeration (Ercolini et al., 2009). The use of cold temperatures, is considered the most effective method of retarding or inhibiting microbial growth in meat products during transportation or storage, besides maintaining product quality and extending shelf life (Al-Jasser, 2012). Therefore, it is extremely important to control and maintain the refrigeration temperature within the acceptable limits to ensure the security and integrity (Zhou et al., 2009).

However, is related in chill-stored meat the development of organoleptic spoilage to microbial consumption of meat nutrients, such as sugars and free amino acids and the release of undesired volatile metabolites. These activities may be performed at low temperatures by psychrotrophic bacteria, compromising the sole effect of temperature as affecting preservation (Ercolini et al., 2009). They can grow at low temperatures, modifying their cytoplasmic membrane and increasing the unsaturated fatty acids levels, which keep this membrane in semifluid state, thereby facilitating the transport of nutrients and enzymes (Madigan et al., 2010). The ability of these microorganisms to grow at low temperatures is one of the challenges to the meat industry in relation to meat quality and public health control (Hernández-Macedo et al., 2011).

This decomposition is largely caused by psychrotrophic species *Brochothrix thermosphacta* which represents a significant component of microbial deterioration of meat. For this micro-organism, the meat is a growth medium which can do it both aerobically

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[1]*Departamento de Engenharia de Alimentos, Instituto de Ciências Tecnológicas e Exatas, Universidade Federal do Triângulo Mineiro – UFTM, Uberaba, MG, Brasil*
[2]*Departamento de Ciência dos Alimentos, Universidade Federal de Lavras – UFLA, Lavras, MG, Brasil*
[3]*Departamento de Ciência da Computação, Universidade Federal de Lavras – UFLA, Lavras, MG, Brasil*
*Corresponding author: leticiauftm@gmail.com

and in anaerobic conditions, producing strong odors (Pin et al., 2002) associated with the production of acetoin, diacetyl and 3-methylbutanol (Dainty & Mackey, 1992).

A recent study by Nowak & Piotrowska (2012) on the microbial degradation of *B. thermosphacta* in meat and meat products, showed that different strains produce different hydrolases which degrade meat. Some strains are capable of degrading protein, or even produce proteases with different substrate specificities. Casaburi et al. (2014) studied the activity of many strains of *B. thermosphacta* in meat reported that almost all were able to grow in the presence of sarcoplasmic extract with glucose and produce histamine, which is used as a quality assessment criterion.

To assess the quality of the meat during storage or refrigerated transportation, if it was initially contaminated with *B. thermosphacta*, we can use the predictive microbiology. This tool aims to provide reliable models for microbial behavior simulations in food products (Couvert et al., 2010). Therefore, the use of predictive models can have a very effective application in the food industry, providing reliable predictions able to prevent risks to consumer health in addition to making the process economically viable, by diminishing losses and helping in decision-making (Juneja et al., 2003).

This work was done in order to model and validate mathematical models that describe the growth of *Brochothrix thermosphacta* at different temperatures and pH.

2 Materials and methods

2.1 Standardization and maintenance of the inoculum

The lyophilised bacteria *Brochothrix thermosphacta* ATCC 11509 was used in the experiment and it was stored in freezing media (15 mL of glycerol, 0.5 g bacteriological peptone, 0.3 g yeast extract, 0.5 g NaCl and 100 mL of distilled water) in freezer at -18 °C.

To use the strains, they were activated into BHI broth and incubated at 28 °C/ 24 h to obtain the number of cells necessary for standardization.

2.2 Effect of storage temperature and pH of the medium on growth of B. thermosphacta in meat broth

Suitable aliquots of standard inoculum were transferred to 100 mL of meat broth (10 g meat extract, 10 g peptone, meat, 5 g tryptone and 5 g glucose / 1 L) at a final concentration of 10^4 CFU/mL and incubated at temperatures of 4 °C, 7 °C and 12 °C. The pH of culture medium was initially adjusted to 5.5; 6.0 and 6.3 with 2M NaOH and 2M HCl at pH meter (model Digimed DM20).

The growth of *B. thermosphacta* in each pH and temperature was monitored at these times: 3 hours, 6 hours, 9 hours, 12 hours, 24 hours, 30 hours, 36 hours, 48 hours, 54 hours, 60 hours, 72 hours, 84 hours, 96 hours, 108 hours and 120 hours, in which 1 mL of aliquot was collected and transferred to tubes containing 9 mL of 0.1% peptone water, performing serial dilutions. Aliquots of 0.01 mL of appropriate dilutions were incubated on plates containing TSA, by using the technique of the microdrop. The plates were incubated at 28 °C/24 hours, and then had their colonies quantified.

2.3 Analysis of growth data for obtaining models

In the first stage, the maximum specific growth rate (μ_m), and the lag phase (λ) were calculated for each experimental combination. Growth parameters were obtained by the modified Gompertz and Baranyi and Roberts equation to the experimental data through DMFit 3.0 program. In the second stage, the estimates obtained for μmax were adjusted for the extended Ratkowsky model to determine the effect of temperature and pH on the maximum specific growth rate of *B. thermosphacta,* according to the following Equation 1:

$$\mu_{\max} = a\left(pH - pH_{mim}\right)\left(T - T_{mim}\right)^2 \tag{1}$$

where a is the regression constant, and pHmim, Tmim are respectively the minimum pH and minimum temperature theoretically estimated for microorganism growth.

2.4 Validation of the results by statistical analysis of models

The following statistical parameters were calculated for validation of the models: correlation coefficient (R^2), mean square error (RMSE), bias factor and accuracy factor (Samapundo et al., 2005). The correlation coefficient (R^2) describes the model fit throughout the length of the curve; the closer the value R^2 is to one, the better the model fit is.

The mean square error (RMSE) is given by Equation 2, and presents the model error relative to the data, that is, how the predicted values are close to the observed values; the closer to zero, the better the fit is.

$$RMSE = \frac{SQR}{n} = \Sigma(value_{obs} - value_{pred})^2 \tag{2}$$

where, $value_{obs}$ is the experimental value, $value_{pred}$ is the value predicted by the model, SQR is the sum of square residuals, and n is the number of degrees of freedom (number of data points - number of model parameters).

The bias factor shown in Equation 3 gives the same weight in the average values that overestimates and underestimates the average, that is, an average relative deviation.

$$Bias\ factor = 10^{\Sigma \log\left[\left(value_{obs} - value_{pred}\right)/n\right]} \tag{3}$$

where, $value_{obs}$ is the experimental value, $value_{pred}$ is the value predicted by the model, and n is the number of data points minus the number of model parameters.

The accuracy factor is the most reliable and accurate statistical measurement because it uses both the predicted and observed values, assessing the percentage prediction error. This factor takes into account only the absolute values. The closer the value is to 1, the lower the percentage error is. The calculation factor was corrected by Equation 4.

$$Accuracy\ factor = 10^{\Sigma\left|\log\left(value_{obs} - value_{pred}\right)\right|} / n \tag{4}$$

where, $value_{obs}$ is the experimental value; $value_{pred}$ is the value predicted by the model, and n is the number of data points minus the number of model parameters.

3 Results and discussion

3.1 Primary growth model of Brochothrix thermosphacta in meat broth

Table 1 shows the growth parameters for *B. thermosphacta* at three different temperatures and pH values.

Table 2 shows the coefficient of determination (R^2) and the root mean square error (RMSE) for the primary models.

The equations of Baranyi and Roberts and the modified Gompertz models for growth conditions of *B. thermosphacta* at 4 °C, 7 °C and 12 °C at pH 5.5; 6.0 and 6.3, are shown in Table 3.

For the analysis of data shown in Table 1 note that the lag phase estimated by the Baranyi and Roberts model is very short in the tested conditions, was practically nonexistent at 4 °C and pH 6.0 and 6.3 and at 12 °C and pH 5.5. With regard to the maximum specific growth rate, it presents, at pH 6.0, higher value as much as 4 °C to 7 °C, confirmed by both models applied. This faster growth at pH 6.0 is notable when comparing the population level reached by *B. thermosphacta*. For example, concentration around 10^6 CFU / mL, order in which the deteriorative signs begin to emerge, is reached in about 50 hours at 4 °C and pH 6.0, while in other conditions, pH 5.5 and 6.3, this concentration is reached only about 70 hours. Therefore, it can be inferred that meat pH 5.5 or 6.3, stored at 4 °C, is preserved better than at pH 6.0. How we can see in Figure 1 bellow.

The fact that the growth of *B. thermosphacta* provide short lag phase in all tested conditions was probably due to the culture activation step prior to inoculation into broth.

Table 1. Growth parameters observed for *B. thermosphacta* in meat broth at 4 °C, 7 °C and 12 °C and pH 5.5, 6.0 and 6.3.

Growing conditions	Baranyi e Roberts		Gompertz	
	λ (h)	μ_{max} (h^{-1})	λ (h)	μ_{max} (h^{-1})
4 °C and pH 5.5	4.85	0.036423	-	0.029839
4 °C and pH 6.0	3.24E-07	0.048561	-	0.042477
4 °C and pH 6.3	1.86E-07	0.043232	-	0.035897
7 °C and pH 5.5	13.29	0.065708	11,87	0.060474
7 °C and pH 6.0	10.05	0.078342	8,26	0.070011
7 °C and pH 6.3	6.38	0.073423	-	0.060197
12 °C and pH 5.5	1.41E-06	0.092588	-	0.076627
12 °C and pH 6.0	2.63	0.10998	-	0.090355
12 °C and pH 6.3	1.59	0.112014	-	0.093117

λ: duration of lag phase; $\mu_{max:}$ maximum specific growth rate.

Table 2. R^2 and RMSE of primary growth model for *B. thermosphacta*.

Growing conditions	Baranyi and Roberts		Gompertz	
	R^2	RMSE	R^2	RMSE
4 °C and pH 5.5	0.99	0.1682	0.99	0.1764
4 °C and pH 6.0	0.98	0.1444	0.98	0.1453
4 °C and pH 6.3	0.98	0.1341	0.98	0.1348
7 °C and pH 5.5	0.994	0.1149	0.992	0.1356
7 °C and pH 6.0	0.993	0.1335	0.996	0.1047
7 °C and pH 6.3	0.996	0.1065	0.995	0.1144
12 °C and pH 5.5	0.993	0.1267	0.993	0.1267
12 °C and pH 6.0	0.995	0.1099	0.996	0.0958
12 °C and pH 6.3	0.98	0.1600	0.992	0.1358

Note also, at 7 °C, a decrease in the duration of the lag phase of 13.28 hours to 6.28 hours, as the pH becomes more near neutrality (Table 1; Figure 2). As the μ_{max} parameter, as had already occurred at 4 °C, the highest value was found at pH 6.0. Generally, the pH closer the pH neutral, faster will be microbial growth. Leroi et al. (2012) highlight in their work the neutral pH was great for the growth of *B. thermosphacta*, but its development was possible even in pH 4.8. Corroborating Gribble et al. (2014) which points out that under aerobic conditions *B. thermosphacta* is able to grow at low pH. The Figure 2 bellow shows the prediction curves at 12 °C.

The analysis of the growth parameters at 12 °C, shows the lag phase, estimated only by the Baranyi and Roberts model, very short, particularly in pH 5.5 and μmax increased as the pH rises. The Figure 3 shows the prediction curves at 12 °C.

Table 3. Baranyi and Roberts and modified Gompertz growth models for *P. fluorescens* at 4 °C, 7 °C and 12 °C, and pH 5.5, 6.0, and 6.3.

Model	Growing conditions	Equation
Baranyi and Roberts	4 °C and pH 5.5	dN/dt= -1.1932043x0.036423x [1-N(t)/7.149077]xN(t)
	4 °C and pH 6.0	dN/dt= - 1x0.048561x [1-N(t)/7.701309]xN(t)
	4 °C and pH 6.3	dN/dt= -1x0.043232x [1-N(t)/7.924358]xN(t)
Gompertz	4 °C and pH 5.5	LogN(t)=3.838248 + 3.49116117exp{-exp [-0.023233 (t-43.04189)]}
	4 °C and pH 6.0	LogN(t)=3.810863 + 3.81597145exp {-exp [-0.030258(t-33.04901)]]
	4 °C and pH 6.3	LogN(t)=3.977424 + 4.19993083exp { -exp [-0.02323 (t-43.04189)]}
Baranyi and Roberts	7 °C and pH 5.5	dN/dt=2.3944289x0.065708x [1N(t)/7.978267]xN(t)
	7 °C and pH 6.0	dN/dt=2.3944389x0.078342x [1N(t)/8.258502]xN(t)
	7 °C and pH 6.3	dN/dt=1.5980269x0.073423x [1N(t)/8.353738]xN(t)
Gompertz	7 °C and pH 5.5	LogN(t)=4.137266+3.70408461exp {-exp[0.044379(t-34.40365)]}
	7 °C and pH 6.0	LogN(t)=4.074842+4.05749568exp {-exp[0.046903(t- 29.58479)]}
	7 °C and pH 6.3	LogN(t)=4.063964+4.17958697exp {-exp[0.039151(t- 25.54234)]}
Baranyi and Roberts	12 °C and pH 5.5	dN/dt=1.0000001x0.092588x [1N(t)/8.332531]xN(t)
	12 °C and pH 6.0	dN/dt=-1.3350782x0.10998x [1-N(t)/8.491625]xN(t)
	12 °C and pH 6.3	dN/dt=-1.195696x0.112014x [1-N(t)/8.472856]xN(t)
Gompertz	12 °C and pH 5.5	LogN(t)=4.30574+3.93048849exp {-exp[-0.052994 (t-18.86998)]}
	12 °C and pH 6.0	LogN(t)=4.448302+3.96353344exp {-exp[0.061968(t- 16.13748)]}
	12 °C and pH 6.3	LogN(t)=4.510883+3.88615589exp {-exp[0.065133(t-15.35312)]

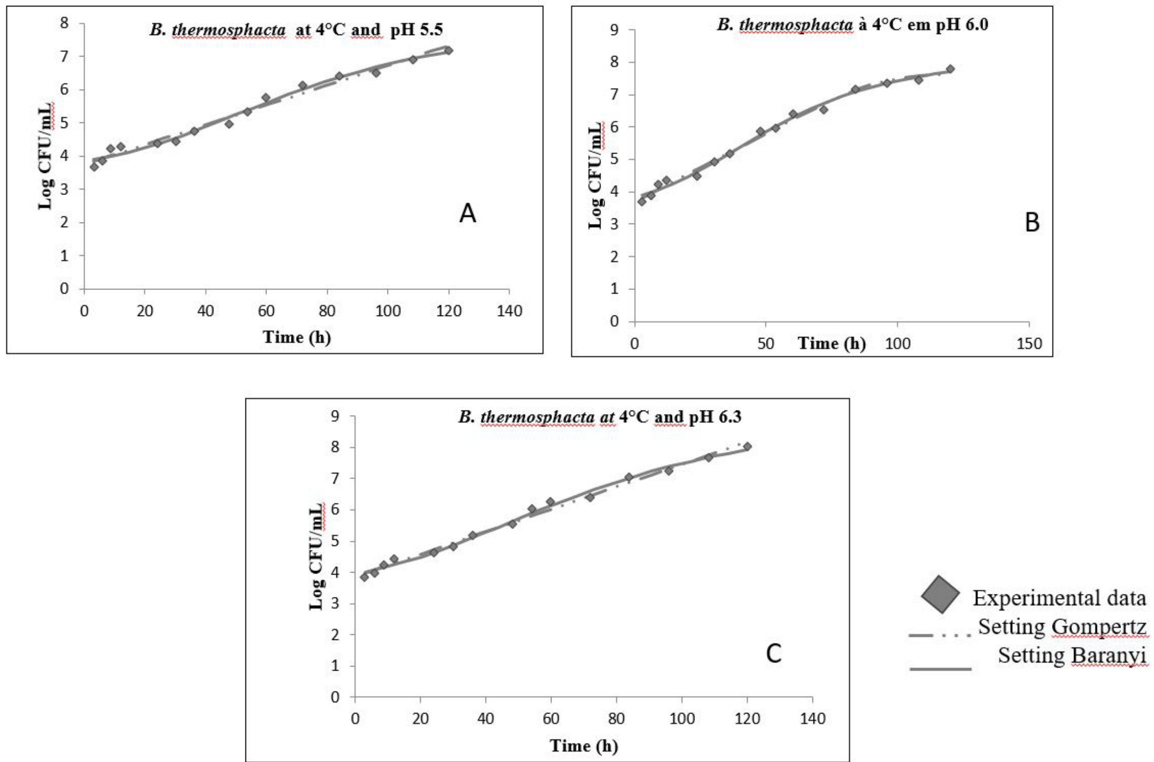

Figure 1. Primary growth modeling of *B. thermosphacta* at 4 °C and pH 5.5 (A), pH 6.0 (B) and pH 6.3 (C).

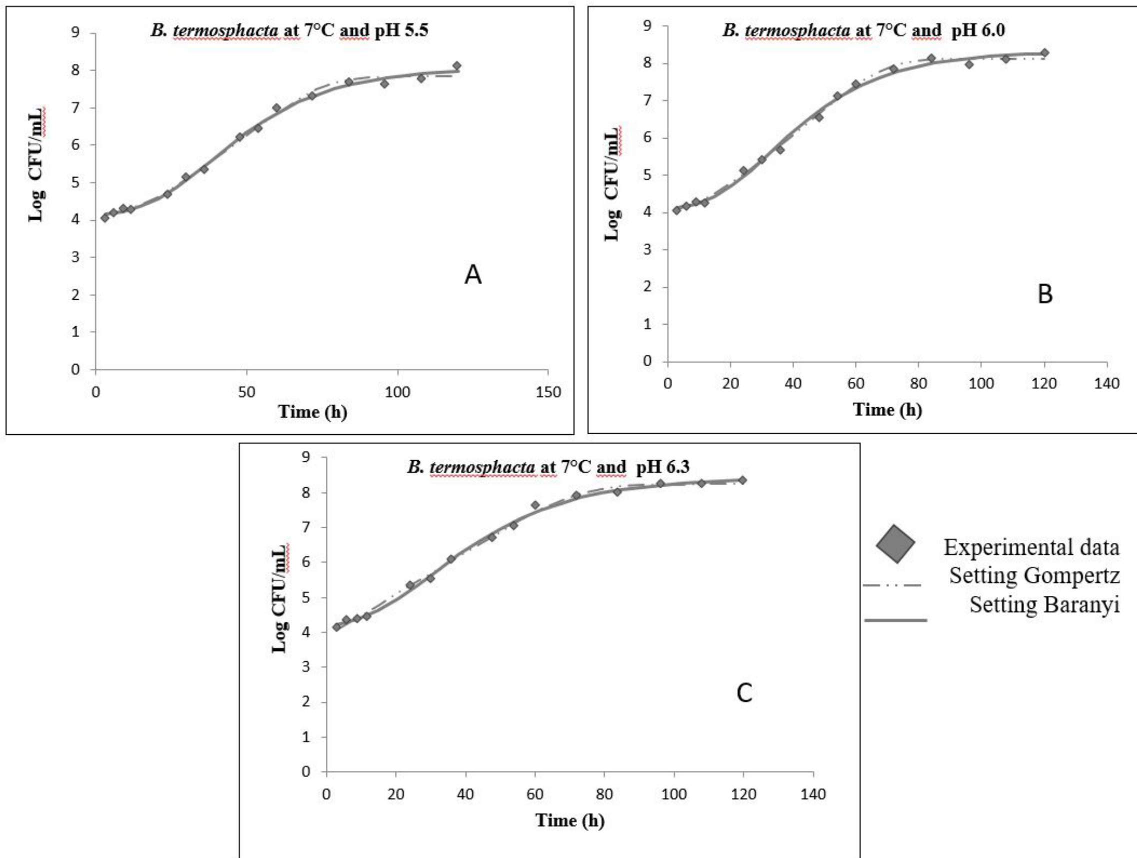

Figure 2. Primary growth model of *P. fluorescens* at 7 °C and pH 5.5 (A), pH 6.0 (B) and pH 6.3 (C).

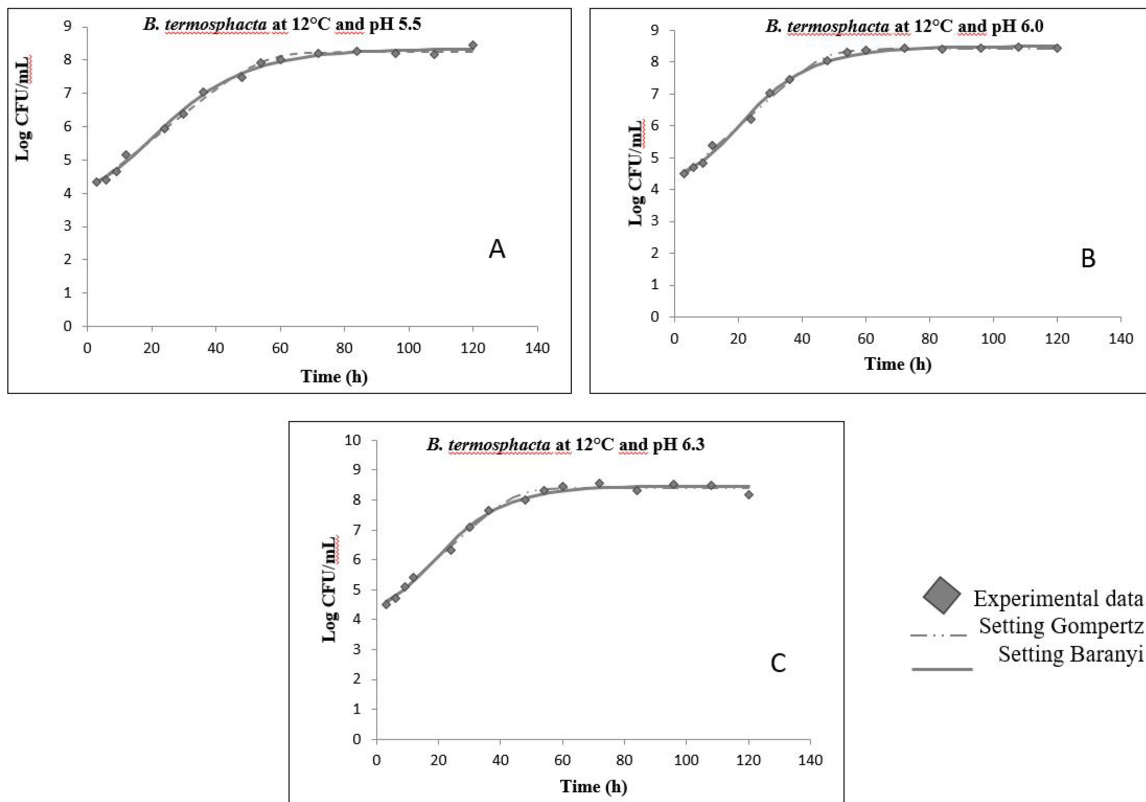

Figure 3. Primary growth model of P. *fluorescens* at 12 °C and pH 5.5 (A), pH 6.0 (B) and pH 6.3 (C).

By analyzing the behavior of *B. thermosphacta* in relation to the temperature rise of 7 °C (ideal for cooling) to 12 °C (temperature abuse), it is observed considerable reduction of lag phase of 13.28h; 10.05h and 6.38h to 1.41*10^{-6}h; 2.36h and 1.59h, at pH 5.5; 6.0 and 6.3 respectively. It is also noted an increase in the maximum specific growth rate of 0.065708 h^{-1}, 0.078342 h^{-1} and 0.073423 h^{-1}, at pH 5.5; 6.0 and 6.3, respectively, by the Baranyi and Roberts model, to 0.092588 h^{-1}, 0.10998 h^{-1} and 0.112014 h^{-1}, respectively and 0.060474 h^{-1}, 0.070011 h^{-1} and 0.060197 h^{-1}, at pH 5.5; 6.0 and 6.3, respectively, by modified Gompertz model to 0.076627h^{-1}, 0.090355h^{-1} and 0.093117h^{-1}, respectively. The comparison of predicted values for µmax, when the temperature exceeds 4 °C, ideal for cooling, to 12 °C, abuse temperature, it is clear that these rates more than double. It is emphasized, therefore, the importance of using low temperatures to delay the onset of changes that occur in the meat due the development of spoilage bacteria (Ercolini et al., 2009).

The comparison of all growth conditions applied to *B. thermosphacta* shows that as the temperature is high µmax also raises, and that pH increase resulted in increased µmax only at 12 °C, since in the other tested temperatures, it was noted that growth in the intermediate pH tested (6.0) showed higher µmax and the pH increase also resulted in a decrease in λ, in two temperature conditions (4 °C and 7 °C).

Regarding λ, there was no direct correlation between pH and temperature for this parameter. It is only noted that the increase of 4 °C to 7 °C led to increase in length of the lag phase of 4.85h 3.24*10^{-7}h and 1.86*10^{-7}h at pH 5.5; 6.0 and 6.3, to 13.29h, 10.05h and 6.38h respectively, while the rise in pH at each of these temperatures to the reduction of lag phase as pH approaches neutrality.

It can be said that the λ and µmax values estimated by the primary growth models are considered valid, since, for all growing conditions and for the two tested models showed R^2 very close to 1 and RMSE near zero (Table 2). This means that the curves of Baranyi and Roberts and modified Gompertz adjusted well to the experimental data and the predicted values are close to those observed. In general, the Baranyi and Roberts model showed slightly better fit to the experimental data.

Once validated, the obtained primary equations (Table 3) can be used to predict *B. thermosphacta* growth in meat broth, in the same conditions tested.

3.3 Secondary growth model of B. thermosphacta in meat broth

Table 4 shows the secondary models generated for the effect of temperature and pH on the maximum specific growth rate and statistical parameters for validate, coefficient of determination (R^2), the root mean square error (RMSE), the bias factor and the accuracy factor, to validate the growth models of *B. thermosphacta* in broth

By means of the equations generated by this modeling (Table 4) can be varied combinations of temperature and

Table 4. Secondary models for *B. thermosphacta* and statistical parameters for validate.

Primary model	Secundary model of the square root	R^2	EQM	Bias factor	Accuracy factor
Baranyi and Roberts	μ_{max}=4.61577*10^{-5}[(T- 10.977245)]^{2}x(pH- 1.5099520)	0.9323	0.0136	0.9982	1.3845
Gompertz	μ_{max} = 0.0000367[(T -(-11.7047))]2x(pH – 1.567511)	0.8630	0.0175	0.9971	1.0799

pH values and estimate the maximum specific rate at which *B. thermosphacta* grow in broth or meat or fresh meat, since the meat broth simulates the nutritional requirements for fresh meat. For example, meat with an initial pH of 5.6 and stored at 4 °C, by Baranyi and Roberts model, present μmax of 0.04234h^{-1}. Already meat with an initial pH of 6.2 and stored at 9 °C present μmax of 0.08639h^{-1}. Note, through these values, that the increase of pH and temperature cause greatly impacts in the μmax lifting. This same relationship was observed by Koutsoumanis et al. (2006) in a study with fresh ground beef with pH ranging between 5.34 and 6.13 and stored at different temperatures (0-20 °C). Similarly Leroi et al. (2012) in a study with different strains of B. *thermosphacta* observed increase in μmax. with the temperature rise (10-35 °C) and also with increasing pH 4.8 to around 7.0, above this value was noted to drop in μmax.

By analyzing the statistical parameters, the two models can be considered validate, but overall the secondary model generated from the primary model of Baranyi and Roberts presents better fit of Ratkowsky model to experimental data, shows slight advantage as the validation of the generated secondary equations

4 Conclusion

The modeling of the growth of *B. thermosphacta* in meat broth showed good agreement of the curves of the primary models to experimental data and low deviation between observed and predicted values, which can then be used to predict the growth of *B. thermosphacta* under the same conditions tested. It was noted that the pH changes did not impact much on growth parameters such as temperature changes.

Finally, the secondary models generated showed good statistical indices (R^2, RMSE, bias factor and factor accuracy) for both primary models that generated them, which makes them validated to estimate μmax of *B. thermosphacta*, when the temperature and pH varies.

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