Elsevier Editorial System(tm) for Journal of Theoretical Biology

Manuscript Draft

Manuscript Number: JTB-D-17-00990

Title: Comparison among growth model of interacting microorganism: a lactic acid bacteria and Listeria monocytogenes

Article Type: Regular paper

Keywords: predictive food microbiology, interacting bacteria, food preservation

Corresponding Author: Dr. Carlos Enrique Schvezov, PhD

Corresponding Author's Institution: Institute fro Materials of Misiones - IMAM

First Author: Carlos Enrique Schvezov, PhD

Order of Authors: Carlos Enrique Schvezov, PhD; Alejandro H Pedrozo, Chemical Eng.; Andrea M Dallagnol, PhD.

Abstract: Predictive food microbiology is normally based on mathematical models to predict the growth, inactivation or probability of microorganism growth which can be applied to establish the shelf-life of food. At present the effort in modeling is oriented towards extrapolation of results beyond experiments in order to predict growth of interacting microorganisms and develop new food preservation processes. In the present report two different mechanistic models which describe the growth of two interacting bacteria such as a lactic acid bacteria and Listeria monocytogenes are developed; they include two new inhibition functions based on kinetic reactions to describe the dynamic behavior of heterogeneous cell population. Both models are easy to handle and permit to introduce other kinetic reactions for more complex scenarios

**1. Cover Letter**

Instituto de Materiales de Misiones
CONSEJO NACIONAL DE INVESTIGACIONES CIENTÍFICAS Y TÉCNICAS
UNIVERSIDAD NACIONAL DE MISIONES



Posadas, October 30, 2017

Dear Editor

I am attaching in the web page of The journal of Theoretical Biology a full paper entittled *"Comparison among growth model of interacting microorganism: a lactic acid bacteria and Listeria"* by Alejandro H. Pedrozo, Andrea M. Dallagnol, Carlos E. Schvezov of the Instituto de Materiales de Misiones (IMAM-UNaM), Misiones, Argentina to be considered for publication in your Journal.

Sincerely,

Carlos Schvezov

Director

Reviewers for:
***Comparison among growth model of interacting microorganism: a lactic acid bacteria and Listeria***
Alejandro H. Pedrozo, Andrea M. Dallagnol, Carlos E. Schvezov

Dey Sutirth
http://www.iiserpune.ac.in/people/faculty-details/20

Zhilan Feng
https://www.math.purdue.edu/people/bio/zfeng/Vita

**J.F. Van Impe**
Chemical and Biochemical Process Technology and Control (BioTeC+), Department of Chemical Engineering, KU Leuven, Gebroeders De Smetstraat 1, B-9000 Gent, Belgium.
jan.vanimpe@cit.kuleuven.be

**F. Pérez-Rodríguez**
Dpt Bromatologia y Tec. de los Alimentos University of Córdoba, Córdoba, Spain.
b42perof@uco.es

**McKellar**
Guelph Food Research Centre, Agriculture and Agri-Food Canada, Guelph, Ontario, Canada N1G 5C9
robin.mckellar@sympatico.ca

**Y. Le Marc**
Institute of Food Research, Norwich Research Park, Norwich NR4 7UA, UK
yvan.lemarc@bbsrc.ac.uk

**Lebert Andre**
INRA Clermont-Ferrand - Theix, Unite´ Qualite´ des Produits Animaux, 63122 Saint-Gene`s Champanelle, France
lebert@clermont.inra.fr

**Christopher Doona**
US Army RDECOM, Natick Soldier Center, Combat Feeding Directorate, Combat Feeding Innovative Science Team, Kansas St., Natick, MA 01760-5018, USA
christopher.doona@natick.army.mil

# Comparison among growth model of interacting microorganism: a lactic acid bacteria and *Listeria monocytogenes*

Alejandro H. Pedrozo, Andrea M. Dallagnol, Carlos E. Schvezov*

Instituto de Materiales de Misiones (IMAM-UNaM), Félix de Azara 1552 (N3300LQD), Misiones, Argentina.

*Corresponding author: email: schvezov@gmail.com. Ph(of.) 54 (376) 449 7141.

**Abstract**

Predictive food microbiology is normally based on mathematical models to predict the growth, inactivation or probability of microorganism growth which can be applied to establish the shelf-life of food. At present the effort in modeling is oriented towards extrapolation of results beyond experiments in order to predict growth of interacting microorganisms and develop new food preservation processes. In the present report two different mechanistic models which describe the growth of two interacting bacteria such as a lactic acid bacteria and *Listeria monocytogenes* are developed; they include two new inhibition functions based on kinetic reactions to describe the dynamic behavior of heterogeneous cell population. Both models are easy to handle and permit to introduce other kinetic reactions for more complex scenarios

**Key words**

modeling, interacting bacteria, food preservation, lactic acid bacteria

## 1. Introduction

Predictive food microbiology is a multidisciplinary field which include disciplines such as mathematics, engineering, chemistry and microbiology to predict microbial behavioral in specific food under defined conditions. Mathematical models incorporate basic and constitutive equations from those fields in order to predict the growth, inactivation or probability of microorganism growth which can be applied to establish the shelf-life of food (Macdonald and Sun, 1999). Lactic acid bacteria (LAB) are starter cultures able to compete with food microorganisms and inhibit or delay growth of food-borne pathogens such as *Listeria monocytogenes* (Vignolo et al., 2012). They can generally exert antimicrobial effect by the production of inhibitory compounds which can either be unspecific metabolites such as acetic acid, phenyllactic acid, indolelactic acid, etc. (Rodríguez-Pazo et al., 2013, Dallagnol et al., 2015); or more complex and specific compounds such as bacteriocins (Alvarez-Sieiro et al., 2016). Consequently, LAB is generally used as bio-preservative agents for controlling *Listeria monocytogenes* and their growth are usually predicted by mathematical models. In this regard, a good fitting model should be able to describe the behavior of both microorganisms with a biological interpretation.

The mathematical models can be classified in three levels; primary, secondary and tertiary models (Whiting and Buchanan, 1993). The primary models consist of mathematical functions which are used to describe the time evolution of the number of cells occurring under specific conditions. In addition, the primary models provide information about the growth parameters of the microorganisms. The secondary models consist of a set of equations which describe the changes of the growth parameters as a function of the environmental conditions, the temperature, the pH and the water activity. The tertiary models consist of user programs which include the primary and secondary models and therefore permit to use them for predictive microbiology.

In particular, the prediction of bacterial growth in food based on primary models must be able to describe growth with as few parameters as possible (McKellar & Lu, 2003). Bacterial growth

normally have three characteristic phases; i) the lag phase in which the bacteria get used to the environment; ii) the exponential growth phase where the biomass growth is the fastest and iii) the stationary phase where growth may even stop.

Several primary models where developed in order to describe the growth phases. For instance, the logistic model (Gompertz) has been modified to describe the growth curves using four parameters (Gibson et al., 1997). The three phases have also been approximated by three linear segments in a so-called tri-linear model (Buchanan et al., 1997). Another model proposed in the literature include four parameters in a logistic type model with delay which has very good fitting capacity (Rosso et al., 1996). This model has been modified and used to describe the simultaneous growth of *Listeria monocytogenes* (LM) and a lactic acid bacteria (LAB) (Gimenez and Dalagerd, 2004). Despite the good fitting capacity of the fully empirical primary models, the model parameters included do not permit to describe the mechanisms by means of which the bacteria get used to the new environment or how is the inhibiting growth process.

A biological interpretation of the lag phase based on a physiological state concept was achieved by incorporation of parameters in a model (Baranyi and Roberts, 1994) which is simple to use, can be used under dynamic conditions, have good fitting capacity and as mentioned before, the parameters have biological meaning (Isabelle and Andre, 2006). Other models have been proposed with similar results which include a heterogeneous population of cells in two phases; no growth and growth; despite their good results they are more difficult to apply (McKellar, 1997, McKellar and Lu, 2003). Heterogeneous population has also been modeled using a deterministic approach, which partially describe part of the whole growth curve (Baranyi 1998; Baranyi 2010).

In the case of simultaneous growth of the interacting bacteria, primary models have been applied (Gimenez and Dalagard, 2004) for LM and LAB with the same limitations of the empirical models which do not describe the growth mechanisms for each phase nor the inhibiting process functions.

This inhibition mechanisms have been incorporated in the simultaneous growth of *staphylococcus aureus* and LAB (Le Marc et al., 2009), as an interacting parameter related to

the population of LAB adapting the models of Baranyi and Roberts (1994) and Gimenez and Dalgard (2004). However, the inhibition is associated with the metabolites (acids and bacteriocins) they produce and not directly with the population.

In addition, models do not provide biologically based mechanisms for the growth inhibition of the target bacteria (Van Impe et al., 2005; Poschet et al., 2005). Models applied to the simultaneous growth of LM and LAB including inhibition growth were based on the privation of nutrients on the substrate or the accumulation of metabolites (Van Impe et al., 2005). These models are very flexible, can be applied to the growth of more than one bacteria, have good fitting capacity and the parameters have biological interpretation. However, the inhibiting function does not provide a biological interpretation of the process or mechanisms.

At present the effort in modeling is oriented towards extrapolation of results beyond experiments to predict growth of interacting microorganisms in order to develop new food preservation processes.

In the present report two primary models are proposed to describe the growth of microorganisms for one species alone or two interacting species. The models are based on the deterministic model due to Baranyi (1998) with the incorporation of an inhibition mechanism based on series-parallels reactions (Ross et al., 2005). The results are compared with those published in the literature on the interacting *Listeria monocytogenes* and LAB.

## 2. Model Formulation and Equations

Two models are proposed for the growth of one kind of bacteria and then applied for two kinds of interacting bacteria. The difference between the models is the degree of heterogeneity of the population. In the first model or Model I, the bacteria are assumed to be in either one of the two phases; no growth or exponential growth. In addition, a second classification is based on the dynamic state of the bacteria in which only two states are considered: growth and no-growth states. In Model I the two states are similar to the phases. This distinction becomes relevant in

the case of modeling with more than two phases as in the following model. The second model or Model II, considers three phases; lag, exponential growth and stationary phases. The lag and the stationary phases correspond to the no-growth dynamic phase. Both models are schematically shown in Figure 1. The bacteria can go just once from the lag to the exponential growth phase in an irreversible way, when there are favorable conditions to grow. In addition, when the concentration of metabolites is high enough the bacteria may go from the exponential growth phase to the stationary phase in the no growth phase and remain there until the end of the simulation.

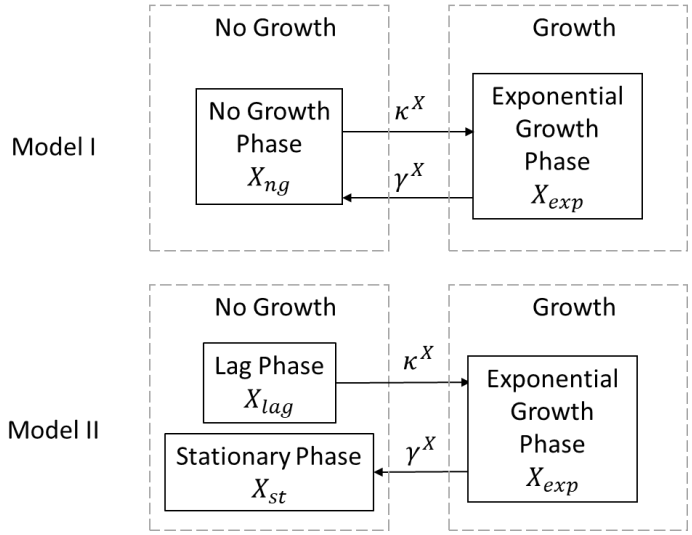


**Figure 1**. Schematic representation of bacteria population and transitions in each model. States in dashed line and phases in solid lines.

## 2.1 Model I

In this model the total population of cells is heterogeneous and composed of some cells in the no growth phase and others in the exponential growth phase. The transition from no growth to the growth phase occurs due to the physiological state and ambient conditions. This transition can be written in a kinetic equation as

$$X_{ng} \xrightarrow{\kappa^X} X_{exp} \quad (1)$$

Where $X_{ng}$ and $X_{exp}$ are the concentration of cells in the no growth and growth phases, respectively; and $\kappa^X$ is the transition rate from the no growth to the growth phase.

Once the cells are in the growth phase, they start to ingest nutrients in order to multiply themselves. In the multiplication process and as a result of the cell division some metabolites are produced and delivered to the environment.

The kinetic equation for the combined process can be written as

$$C + X_{exp} \xrightarrow{\alpha^X} 2\,X_{exp} + \; m\,M^X \quad (2)$$

Where $C$ is the amount of nutrient consumed by the cell, $M^X$ is the metabolite produced by the cells and $\alpha^X$ is the rate of reaction division process.

Most of the reports in the literature consider that the amount of nutrients is limited without renewal as in the case of microorganisms growing in food. In this case growing is a transient state where there is an accumulation of metabolites as by-products, which may be the key factor that stop the growth before the lack of nutrient can affect bacteria multiplication.

So, the metabolites may inhibit growth of bacteria (Van Impe et al., 2005) which affect the growth rate producing a phase change from growth to no growth. This change can be described by the following rate equation

$$X_{exp} + M^X \xrightarrow{\gamma^X} X_{ng} + \; M^X \quad (3)$$

Where $\gamma^X$ is the phase transition rate.

The set of differential equations which describe the growth kinetics of the bacteria for the growth and no growth phases, the production of metabolites and the effect on the bacteria are as follows

$$\frac{dX_{ng}[t]}{dt} = -\kappa^X\left(X_{ng}[t]\right)^{e_1} \quad (4)$$

$$\frac{dX_{exp}[t]}{dt} = \alpha^X(C[t])^{e_2}\left(X_{exp}[t]\right)^{e_3} \quad (5)$$

$$\frac{dX_{exp}[t]}{dt} = \frac{1}{m}\frac{dM^X[t]}{dt} \quad (6)$$

$$\frac{dM^X[t]}{dt} = m\,\alpha^X (C[t])^{e_2} \left(X_{exp}[t]\right)^{e_3} \quad (7)$$

$$\frac{dX_{exp}[t]}{dt} = -\gamma^X \left(X_{ng}[t]\right)^{e_4} (M^X[t])^{e_5} \quad (8)$$

$$\frac{dX_{ng}[t]}{dt} = \gamma^X \left(X_{ng}[t]\right)^{e_4} (M^X[t])^{e_5} \quad (9)$$

Where equation (4) is the kinetic differential equation of the reaction equation (1), in similar way equation (5) corresponds to the reaction equation (2) and equation (8) is associated to the reaction equation (3), for the transition from the growth phase to the no growth phase and equation (9) is the opposite, accounting for the transition of cells to the no growth phase. Equation (6) is the differential equation associated with the production of metabolites in the exponential phase following equation (2) which can be written as equation (7) by using equation(5).

**Table 1.** The full set of parameters and initial conditions

| *Parameter* | *Description* | *Parameter* | *Description* |
|---|---|---|---|
| $e_1$ | Reaction order of $X_{ng}[t]$ | $X_{ng}[0]$ | Initial condition |
| $e_2$ | Reaction order of $C[t]$ | $C[0]$ | Initial condition |
| $e_3$ | Reaction order of $X_{exp}[t]$ | $X_{exp}[0]$ | Initial condition |
| $e_4$ | Reaction order of $X_{ng}[t]$ | $M^X[0]$ | Initial condition |
| $e_5$ | Reaction order of $M^X[t]$ | $\alpha^X$ | Division reaction rate |
| $m$ | Stoichiometric coefficient of $M^X$ | $\kappa^X$ | phase transition rate |
| | | $\gamma^X$ | phase transition rate |

The resultant set of equations (4-9) contains a total of 9 parameters and 4 initial condition, one per each state variable, as listed in Table 1. This number can be reduced to 4 making the following assumptions:

i. The exponents $e_i$ are equal to one

ii. The amount of nutrients is large enough to consider there is no change with time and therefore a new kinetic parameter can be defined as: $\mu^X = \alpha^X C[t]$

iii. The absolute amount of metabolite given by the variable $M^X[t]$ is replaced by a relative amount of metabolite $N^X[t] = M^X[t] / m$ .

iv. The initial population consists of bacteria in the no growth phase, only.

v. Associated with assumption iv), the initial concentration of metabolite is small enough to be considered negligible.

With the assumption i)-v), equations 4-9 are reduced to the following system of equations and initial conditions:

$$\frac{dX_{ng}[t]}{dt} = -\kappa^X X_{ng}[t] + \gamma^X N^X[t] X_{exp}[t] \quad (10)$$

$$\frac{dX_{exp}[t]}{dt} = \mu^X X_{exp}[t] + \kappa^X X_{ng}[t] - \gamma^X N^X[t] X_{exp}[t] \quad (11)$$

$$\frac{dN^X[t]}{dt} = \mu^X X_{exp}[t] \quad (12)$$

$$X_{ng}[0] = x_0 \quad (13)$$

$$X_{exp}[0] = 0 \quad (14)$$

$$N^X[0] = 0 \quad (15)$$

That is; 3 differential equation 10-12, including 3 parameters and 3 initial conditions 13-15, but only one (13) to be specified, as follows:

$x_0$: the initial concentration of bacteria;

$\kappa^X$: the transition rate of bacteria from the no growth to the exponential phase;

$\mu^X$: the growth rate of bacteria in the exponential phase;

$\gamma^X$: the inhibition rate for bacterial growth due to metabolites.

**2.2 Model II**

In this model three phases are considered; lag, exponential growth and stationary. When the conditions are favorable to grow the bacteria in the lag state of the no growth phase can move to the growth phase in an irreversible way; that is, they cannot go back to the lag phase. However, once in the growth phase and when the concentration of metabolites is high enough the bacteria may go to the stationary phase. Moving to the stationary phase is irreversible and therefore they stay in this phase until the end of the simulation, considering that a bacteria death phase is not included in this model. Therefore, this model uses the following kinetic equations to describe the transition between phase of a heterogeneous population and cell division.

$$X_{lag} \xrightarrow{\kappa^X} X_{exp} \tag{16}$$

$$C + X_{exp} \xrightarrow{\alpha^X} 2\,X_{exp} + \; m\,M^X \tag{17}$$

$$X_{exp} + M^X \xrightarrow{\gamma^X} X_{st} + \; M^X \tag{18}$$

Where $X_{lag}$, $X_{exp}$ and $X_{st}$ are the concentration of cells in lag, exponential and stationary phases respectively.

The set of equation for this model can be written as the following differential equations 17-24, including the initial conditions

$$\frac{dX_{lag}[t]}{dt} = -\kappa^X X_{lag}[t] \tag{17}$$

$$\frac{dX_{exp}[t]}{dt} = \mu^X X_{exp}[t] + \kappa^X X_{lag}[t] - \gamma^X\, N^X[t]\, X_{exp}[t] \tag{18}$$

$$\frac{dN^X[t]}{dt} = \mu^X X_{exp}[t] \tag{19}$$

$$\frac{dX_{st}[t]}{dt} = \gamma^X\, N^X[t]\, X_{exp}[t] \tag{20}$$

$$X_{lag}[0] = x_0 \tag{21}$$

$$X_{exp}[0] = 0 \tag{22}$$

$$N^X[0] = 0 \tag{23}$$

$$X_{st}[0] = 0 \tag{24}$$

It is noted that each set of equations 10-15 and 17-24 correspond to two different models for one species in specific environments and assumptions about behavior. However, they can be extended to two species which may be competing in different ways. Next both model approaches are extended to the growth of LAB and LM in the same broth.

## 2.3 Mathematical model for LAB and LM

As mentioned above the growth of LAB and LM has been modeled by different authors (Gimenez & Dalgaard, 2004; Cornu et al., 2011; Mejlholm & Dalgaard, 2015). In general, LAB and LM are assumed to grow in a same nutrient and produce their own metabolites which may inhibit the growth of the other species. However, the effect of the metabolites produced by LAB has a stronger effect in LM than the inverse case, and therefore is the growth of LAB that inhibits the growth of LM and not the other way. Therefore it may be assumed that the metabolites of the LM has negligible effect on LAB and may be neglected in the model (Mejlholm & Dalgaard, 2015).

In the case of the Model I, when applied to the growth of LAB and LM in the same broth, and neglecting the production and effect of metabolites produced by LM on LAB. The ruling equations for both models can be written as follows, including the initial conditions:

### 2.3.1 Model I

$$\frac{dLAB_{ng}[t]}{dt} = -\kappa^{LAB} LAB_{ng}[t] + \gamma^{LAB}\, N^{LAB}[t]\, LAB_{exp}[t] \quad (25)$$

$$\frac{dLAB_{exp}[t]}{dt} = \mu^{LAB} LAB_{exp}[t] + \kappa^{LAB} LAB_{ng}[t] - \gamma^{LAB}\, N^{LAB}[t]\, LAB_{exp}[t] \quad (26)$$

$$\frac{dN^{LAB}[t]}{dt} = \mu^{LAB} LAB_{exp}[t] \quad (27)$$

$$\frac{dLM_{ng}[t]}{dt} = -\kappa^{LM} LM_{ng}[t] + \gamma^{LM}\, N^{LAB}[t]\, LM_{exp}[t] \quad (28)$$

$$\frac{dLM_{exp}[t]}{dt} = \mu^{LM} LM_{exp}[t] + \kappa^{LM} LM_{ng}[t] - \gamma^{LM}\, N^{LAB}[t]\, LM_{exp}[t] \quad (29)$$

Initial conditions

$$LAB_{ng}[0] = LAB[0] \quad (30)$$

$$LAB_{exp}[0] = 0 \quad (31)$$

$$N^{LAB}[0] = 0 \quad (32)$$

$$LM_{ng}[0] = LM[0] \quad (33)$$

$$LM_{exp}[0] = 0 \quad (34)$$

### 2.3.2 Model II

$$\frac{dLAB_{lag}[t]}{dt} = -\kappa^{LAB} LAB_{lag}[t] \quad (35)$$

$$\frac{dLAB_{exp}[t]}{dt} = \mu^{LAB} LAB_{exp}[t] + \kappa^{LAB} LAB_{lag}[t] - \gamma^{LAB}\, N^{LAB}[t]\, LAB_{exp}[t] \quad (36)$$

$$\frac{dN^{LAB}[t]}{dt} = \mu^{LAB} LAB_{exp}[t] \quad (37)$$

$$\frac{dLAB_{st}[t]}{dt} = \gamma^{LAB}\, N^{LAB}[t]\, LAB_{exp}[t] \quad (38)$$

$$\frac{dLM_{lag}[t]}{dt} = -\kappa^{LM} LM_{lag}[t] \quad (39)$$

$$\frac{dLM_{exp}[t]}{dt} = \mu^{LM} LM_{exp}[t] + \kappa^{LM} LM_{lag}[t] - \gamma^{LM}\, N^{LAB}[t]\, LM_{exp}[t] \quad (40)$$

$$\frac{dLM_{st}[t]}{dt} = \gamma^{LM}\, N^{LAB}[t]\, LM_{exp}[t] \quad (41)$$

Initial conditions

$$LAB_{lag}[0] = LAB[0] \quad (42)$$

$$LAB_{exp}[0] = 0 \quad (43)$$

$$N^{LAB}[0] = 0 \quad (44)$$

$$LAB_{st}[0] = 0 \quad (45)$$

$$LM_{lag}[0] = LM[0] \quad (46)$$

$$LM_{exp}[0] = 0 \quad (47)$$

$$LM_{st}[0] = 0 \quad (48)$$

## 2.4 Methods of solution

Each set of equation 25-34 and 35-48 are systems of first order nonlinear ordinary differential equations which are solved applying numerical methods of solutions using the Wolfram Mathematical 9.0 software package. For each species of microorganisms there are four parameters which must be determined as follows: $LAB[0], \mu^{LAB}, \kappa^{LAB}, \gamma^{LAB}, LM[0]$, $\mu^{LM}, \kappa^{LM}, \gamma^{LM}$.

In order to determine the 8 parameters it is used a genetic algorithm of the type NSGA 2 (Deb et al. 2002), which minimizes simultaneously the square errors (SE) between the experimental and calculated values of the growth curves, as described in previous reports. (Pedrozo et al., 2015a; Pedrozo et al., 2015b).

# 3. Results

## 3.1 The genetic algorithm

In order to compare the results between models I and II the results of each model were best fitted with the experimental data of specific population evolution of the interacting LAB

(Tas5612, Tas5610) and LM species (Tas5611, Tas5609) obtained from Combase (www.combase.cc) and produced in the Tasmanian Institute of Agriculture (Australia).

**Table 2**. Results of fit for model I and model II

| Parameters | Units | Model I | | Model II | |
|---|---|---|---|---|---|
| | | Figure 1a | Figure 1b | Figure 2a | Figure 2b |
| $\mu^{LAB}$ | ($h^{-1}$) | 0.1441 | 0.1597 | 0.1326 | 0.1660 |
| $\kappa^{LAB}$ | Log ($h^{-1}$) | -0.9422 | -2.2967 | -0.7035 | -2.4522 |
| $\gamma^{LAB}$ | Log(mL $CFU^{-1}$ $h^{-1}$) | -8.5445 | -7.6581 | -9.5161 | -7.8135 |
| $LAB_0$ | Log(CFU/mL) | 3.19 | 3.05 | 5.9148 | 3.15 |
| $\mu^{LM}$ | ($h^{-1}$) | 0.0940 | 0.1023 | 0.0956 | 0.0879 |
| $\kappa^{LM}$ | Log ($h^{-1}$) | -1.7481 | -2.7619 | -1.7141 | -2.4997 |
| $\gamma^{LM}$ | Log(mL $CFU^{-1}$ $h^{-1}$) | -8.8571 | -8.2124 | -8.9889 | -8.5011 |
| $LM_0$ | Log(CFU/mL) | 2.84 | 2.96 | 6.3815 | 2.78 |

### 3.1.1 Results of Model I

The results of the model I and the referred experimental data for the concentrations of LAB and LM are shown in Figure 2. The parameters in each case were determined using the genetic algorithm described above. In Figures 2a and 2b, it can be clearly distinguished the no growth and growth phases and the smooth transitions obtained with the model. These results using two different experimental results are a test, in particular of the assumptions with respect to both; the correctness of the simplification made on the differential equations and on the other hand, the good performance of the inhibition term for the growth of LM, as proportional to the concentration of metabolites produces by the LAB. It is noted the low quadratic errors for the

LM of around 0.25 for the first case in Figure 2a and 0.26 for the second case in Figure 3a, and also for the case of LAB of 0.22 in Figures 1a and 1.27 Figures 2a.

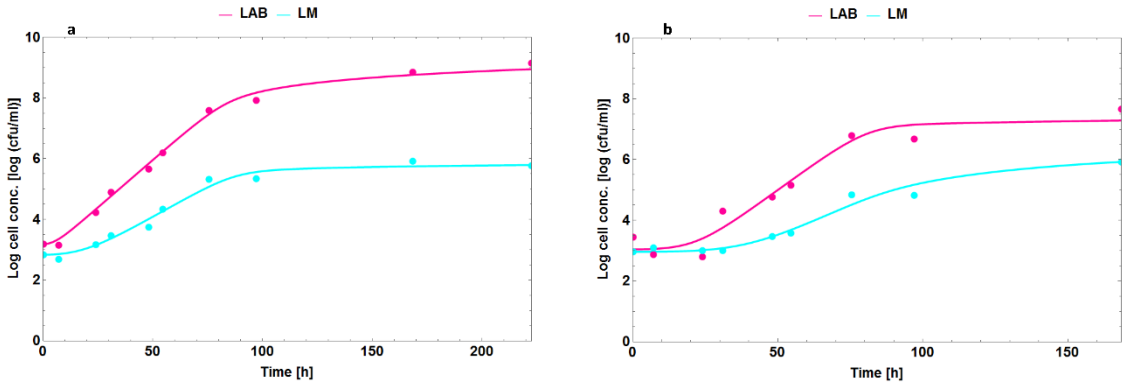


**Figure 2**. Growth curves fitted with model I. a) LAB Tas5612 and LM Tas5611, b) LAB Tas5610 and LM Tas5609

If instead of the total concentration of bacteria in each phase; the concentrations of the no growth and growth phase are considered, the evolution is as follows. In the case of lactic acid bacteria the concentration in the no growth and growth phases are as shown in Figure 3a (Tas5612) and 3b (Tas5610) for each set of experimental data considered. In Figure 3a the concentration of LAB in the no growth phase decreases during the first 30 hours and after this it increases with a high slope up to the 90 hours and then it slowly grows until the end of the simulation time at 200 hours. The initial lag time is relatively short and is the results of the relatively large value of $\kappa^{LAB}$ responsible of the initial growth step. At about 30 h the exponential growth of the cells in the no growth phase would be associated to the increasing concentration of metabolites inhibiting their growth which decreases after 90 hours.

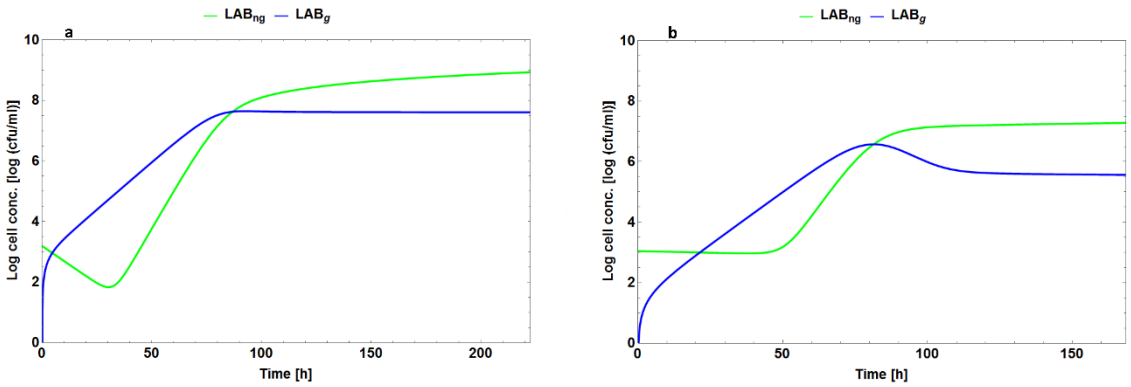


Figure 3: Dynamic behavior of no growth and growth phases predicted with model I. a) LAB Tas5612, b) LAB Tas5610.

In the case of the LAB in the growth phase, in the Figure 3a it is observed that the growth rate is very high up to a time of 80 h where the concentration achieves a maximum value near $10^8$ CFU/ml and then there is a sharp but small change of slope to a negative value for the remaining time of the simulation period in which the concentration decreases to $10^{7.5}$ CFU/mL in the next 140 h.

It is noted that in the exponential growth phase the cell concentration does not tend to zero but tends to a high constant value. This pseudo asymptotic behavior is attributed to the effect of: i) the kinetic equation (1) which produce a constant rate of transfer of bacteria from the no growth to the growth phase; ii) the kinetic equation (3) which provide a constant rate of production of cells in the no growth phase due to the increase in the concentration of metabolites and iii) the absence of a kinetic of death of bacteria.

On the other hand, the evolution of lactic acid bacteria in the no growth and growth phases for the conditions corresponding to Figure 2b for the second set of experimental values are as follows. The lactic acid bacteria concentration in the no growth phase smoothly decreases up to 45 h and then start to grow exponentially for the next 35 h and after this the concentration remains practically unchanged showing a strong inhibition effect. It is observed an initial slow decrease comparing to the evolution of Figure 3a where there is a strong initial decrease. This difference is associated to a smaller value of $\kappa^{LAB}$ in the results shown in Figure 2b, with

respect to the $\kappa^{LAB}$ value listed in Table 2, in the growth conditions resulting in Figure 2a. The exponential change of this phase near 45 h is due to the considerably increase of the concentration of metabolites, producing a high rate transition from growth to no growth phase (eq. 9).

Comparing now the evolution of bacteria in the growth phase in Figure 3b with respect to that in Figure 3a, it is observed that the evolutions are similar up to the time of 80 h when both reach maximum values, however in Figure 3b after the maximum is reached it starts to decrease with a larger rate or slope and then at about 100 h stabilizes at a constant values of about $10^{6.5}$ CFU/ml. This could also be attributed to a smaller value of the rate $\kappa^{LAB}$.

### 3.1.2 Results of Model II

The results of model II applied to the same two experimental growth results used for Model I; a) LAB Tas5612 and LM Tas5611 and b) LAB Tas5610 and LM Tas5609, are shown in Figure 4a and 4b. In Figure 3a the best fit show quadratic errors of 0.4467 for LAB and 0.3041 for LM. The slight larger errors could be associated to the less smooth transition from the exponential to the stationary phase as compared to the case of Model I. The results in Figure 4b show a larger quadratic error for the case of the LAB of 1.3799 as in the case of Model I which is associated to the large noise in the experimental data as can be seen in Figure 4b. For the case of the LM there is a better fitting with a small quadratic error of 0.2792.

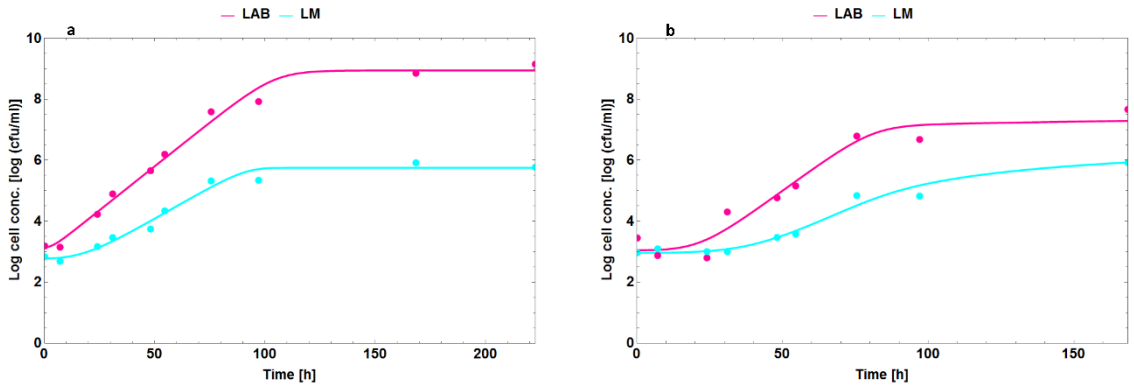


Figure 4: Growth curves fitted with model II. a) LAB Tas5612 and LM Tas5611, b) LAB Tas5610 and LM Tas5609

When the concentration of lactic bacteria in each phase is considered, the results are as observed in Figure 5a and 5b.

In this model there are three phases, lag, exponential and stationary. In the first case of Figure 5a it is observed that the BAL sharply decreases in 40 h to a negligible value. The different behavior respect to Model I is the existence of two phases to describe the no growth behavior in addition to the exponential phase to which the bacteria from the lag phase can go depleting the concentration of the bacteria in the lag phase in 40 h. The cells in the exponential phase start to grow from the beginning at a high rate from about $10^{2.5}$ to $10^{8}$ CFU/mL in about 100 h reaching the maximum concentration and sharply decreasing to the initial concentration in the following next period of time of 100 h. This behavior could be closer to the real situation considering that cells in the exponential phase should disappear at long time. The cell in the stationary phase starts to appear soon in the calculations after 40 h of modeling time. Then the concentration increases at a high exponential rate in the following 60 h and then the rate slows down sharply to a negligible grow rate that remains to the end of the simulation. The evolution of the concentration in lag and exponential phases indicate that for longer periods of time all the cells will be in the stationary phase.

The results for running conditions b are shown in Figure 5b. In the first case, the lag phase shows a very slow growth rate in agreement with the long growing time in the lag phase

observed in Figure 4b, consistent with the small value of the rate parameter $\kappa^{LAB}$. The behavior of the exponential phase is similar to that observed in Figure 5a, showing a sharp increase to a maximum at 85 h and then a symmetric decrease to the initial concentration taking approximately 85 h each step, instead of 100 h as in the first case of Figure 5a. The same pattern in the evolution in both running cases is consistent with what is expected of a decrease in the growth due to the constant production of metabolites.

The evolution of the cells in the stationary phase is similar to that shown in Figure 5a, with cells starting to appear after 40 h of model running time, growing exponentially in the following 35 h and decreasing the growth rate to a negligible value due to the depletion of cells in the exponential phase. After 100 h, the cells in the stationary phase remains constant to the end of the simulation.

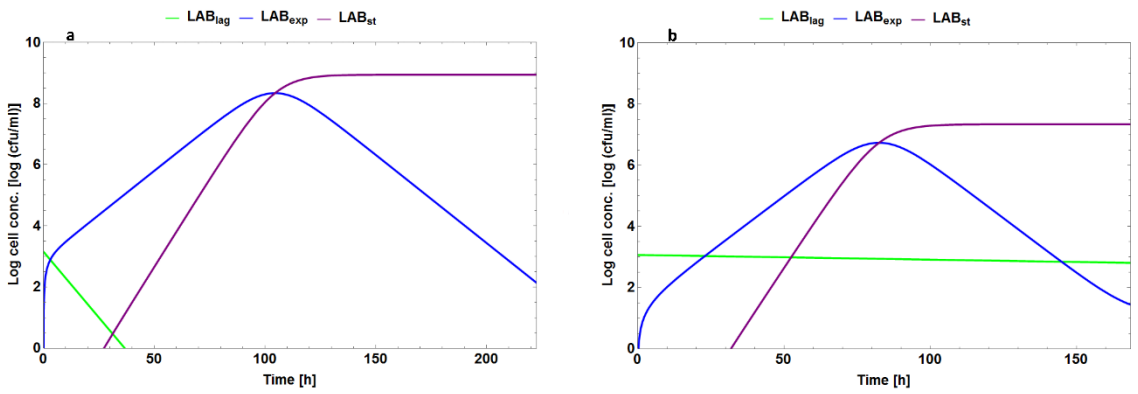


Figure 5: Dynamic behavior of lag, exponential and stationary phases. a) LAB Tas5612, b) LAB Tas5610

### 3.2 Comparison of errors among models

In order to determine the fitting capacity of the different models whose results were presented and analyzed in the previous section, the square correlation coefficient between the experimental data and the different curves obtained with each model were calculated (Table 3).

In addition, these values of square correlation coefficient are compared with model results reported in the literature (Baranyi and Roberts, 1994) for the same experimental data. From the values of errors in Table 3 it is observed that model II and the Baranyi and Roberts model fit the experimental data with similar quality. Moreover, the best fitting is given by model I with the lowest errors. It is noted that the numerical effort and mathematical complexity results low and similar for these three models since they use only 4 parameters.

From the biological point of view and considering that in Model I the concentration of cells in the exponential phase cannot be zero at any time, this model predicts a constant growth which rate decreases with time. On the other hand, Model II predicts a cell growth in the exponential phase reaching a maximum population and then decreasing monotonically, at the same time all the cells go to the stationary phase which may be attributed to the resulting high concentration of metabolites. Therefore, from this analysis, it may be concluded that comparing the Models I and II, the first produce a better fitting however Model II produce results which have a biological interpretation closer to what it may be expected due to the interaction between the LAB and LM.

**Table 3**. Square correlation coefficient ($R^2$) for proposed models and Baranyi and Roberts model

| | $R^2$ | | |
|---|---|---|---|
| Data set | Model I | Model II | Baranyi and Roberts (1994) |
| LAB Tas5612 | 0.9958 | 0.9900 | 0.986 |
| LM Tas5611 | 0.9826 | 0.9779 | 0.970 |
| LAB Tas5610 | 0.9499 | 0.9468 | 0.912 |
| LM Tas5609 | 0.9727 | 0.9698 | 0.962 |

## 4. Summary and conclusions

Two different mechanistic models which describe the growth of two interacting bacteria such as BAL and LM are developed, which include two new inhibition functions based on kinetic reactions among the cells coexisting in different phases at the same time. Both bacteria grow in the same medium. The models were applied to data available in the literature (Tasmania Institute) and the main conclusions are:

- In Model I the cells can be in only two phases; no growth and growth, and the transition from one to other is reversible.
- In Model II the cells can be in three phases lag, exponential and stationary and the transition from one to the other is irreversible, that is these cells which pass to the new stationary phase remains there to the end of the simulation since there is no dead phase.

Model I fits better with the experimental results, however model II provide results with biological meaning. As a result model I provide better data interpolation predictions of the data and on the other hand, Model II may be more useful and reliable for extrapolation of the results. Both models are easy to handle in order to introduce other kinetic reactions which may be considered important for the case under study. For instance, a death kinetic of bacteria in the lag or stationary phase accounting for the effect in the growth curves of the microorganisms.

## 5. Acknowledgements

This work was supported by the Comite Ejecutivo de Desarrollo e Inovación Tecnologica (CEDIT), Misiones, Argentina; the Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Argentina; and the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), Argentina [PICT No. 1746, 2015].

## 6. References

Alvarez-Sieiro, P., Montalbán-López, M., Dongdong, Mu., Oscar P. 2016. Kuipers Bacteriocins of lactic acid bacteria: extending the family. Appl. Microbiol. Biotechnol. 100, 2939-2951.

Baranyi, J. 1998. Comparison of stochastic and deterministic concepts of bacterial lag. J. Theor. Biol. 192(3), 403-408.

Baranyi, J. 2010. Modelling and parameter estimation of bacterial growth with distributed lag time. PhD Thesis. Work for the Institute of Food Research, UK. For. http://www2.sci.u-szeged.hu/fokozatok/PDF/Baranyi_Jozsef/Disszertacio.pdf (accessed 13 March 2003).

Baranyi, J., Roberts, T.A. 1994. A dynamic approach to predicting bacterial growth in food. Int. J. Food Microbiol. 23 (3-4), 277-294.

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 Microbiol. 14 (4), 313-326.

Dallagnol, A.M., Pescuma, M., Rollán, G., Torino, M.I., de Valdez, G.F. 2015. Optimization of lactic ferment with quinoa flour as bio-preservative alternative for packed bread. Appl. Microbiol. Biotechnol. 99, 3839-3849.

Deb, K., Pratap, A., Agarwal, S., Meyarivan, T.A.M.T. 2002. A fast and elitist multiobjective genetic algorithm: NSGA-II. Evolutionary Computation, IEEE Transactions on, 6 (2), 182-197.

Gibson, A.M., Bratchell, N., Roberts, T.A. 1987. The effect of sodium chloride and temperature on the rate and extent of growth of *Clostridium botulinum* type A in pasteurized pork slurry. J. Appl. Bacteriol. 62(6), 479-490.

Gimenez, B., Dalgaard, P. 2004. Modelling and predicting the simultaneous growth of *Listeria monocytogenes* and spoilage micro-organisms in cold-smoked salmon. J. Appl. Microbiol. 96(1), 96-109.

Isabelle, L., André, L. 2006. Quantitative prediction of microbial behaviour during food processing using an integrated modelling approach: a review. Int. J. Refrig. 29(6), 968-984.

Le Marc, Y., Valík, L., & Medveďová, A. 2009. Modelling the effect of the starter culture on the growth of *Staphylococcus aureus* in milk. Int. J. Food Microbiol. 129(3), 306-311.

McDonald, K. , Sun D. 1999. Predictive food microbiology for the meat industry: a review. Int. J. Food Microbiol. 52, 1–27.

McKellar, R. C. 1997. A heterogeneous population model for the analysis of bacterial growth kinetics. Int. J. Food Microbiol. *36*(2), 179-186.

McKellar, R. C., Lu, X. 2003. Modeling microbial responses in food. CRC Press. London, New York.

Mejlholm, O., Dalgaard, P. 2015. Modelling and predicting the simultaneous growth of *Listeria monocytogenes* and psychrotolerant lactic acid bacteria in processed seafood and mayonnaise-based seafood salads. Food Microbiol. 46, 1-14.

Cornu, M., Billoir, E., Bergis, H., Beaufort, A., Zuliani, V. 2011. Modeling microbial competition in food: Application to the behavior of *Listeria monocytogenes* and lactic acid flora in pork meat products. Food Microbiol, 28(4), 639-647.

Pedrozo, H.A., Dallagnol A.M., Schvezov, C.E. (2015) Ingeniería inversa en modelos de crecimiento usando algoritmos genéticos. Latin American Conference on Mathematical Modeling of Biological Systems. CABA, Argentina.

Pedrozo, H.A., Dallagnol A.M., Schvezov, C.E. 2015. Preservación de carne de pescado de agua dulce mediante la interacción de bacterias lácticas y alterantes/patógenos: modelización. Progress report. Scholarship of the Comité Ejecutivo de Desarrollo e Innovación Productiva (CEDIT).

Pérez-Rodríguez, F., Valero, A. 2013. Predictive microbiology in foods. In: Hartel, R.W., Clark, J.P., Finley, J.W., Rodriguez-Lazaro, D., Topping, D. (Eds.), Predictive microbiology in foods. Springer, New York, pp. 1-10.

528 Poschet, F., Vereecken, K. M., Geeraerd, A. H., Nicolaï, B. M., Van Impe, J. F. 2005. Analysis
529 of a novel class of predictive microbial growth models and application to coculture
530 growth. Int. J. Food Microbiol., 100(1), 107-124.
531 Rodríguez-Pazo, N., Vázquez-Araújo, L., Pérez-Rodríguez, N., Cortés-Diéguez, S., Domínguez,
532 J.M. 2013. Cell-free supernatants obtained from fermentation of cheese whey
533 hydrolyzates and phenylpyruvic acid by *Lactobacillus plantarum* as a source of
534 antimicrobial compounds, bacteriocins, and natural aromas. Appl. Biochem. Biotechnol.
535 171, 1042-1060.
536 Ross, E. W., Taub, I. A., Doona, C. J., Feeherry, F. E., Kustin, K. 2005. The mathematical
537 properties of the quasi-chemical model for microorganism growth–death kinetics in
538 foods. Int. J. Food Microbiol., 99(2), 157-171.
539 Rosso, L., Bajard, S., Flandrois, J. P., Lahellec, T. C., Fournaud, J., Veit, P. 1996. Differential
540 growth of Listeria monocytogenes at 4 and 8 C: consequences for the shelf life of chilled
541 products. J. Food Protec., 59(9), 944-949.
542 Tasmanian Institute of Agriculture, University of Tasmania, Australia.
543 Van Impe, J.F., Poschet, F., Geeraerd, A.H., Vereecken, K.M. 2005. Towards a novel class of
544 predictive microbial growth models. Int. J. Food Microbiol., 100(1), 97-105.
545 Vignolo, G., Saavedra, L., Sesma, F., Raya, R., 2012. Food Bioprotection: lactic acid bacteria as
546 natural preservatives. In: Bhat, R., Alias, K.A., Paliyath, G. (Eds.), Progress in Food
547 Preservation. Willey-Blackwell: Wst Sussex, UK, pp. 453-483.
548 Whiting, R.C. Buchanan, R.L. 1993. A classification of models for predictive microbiology.
549 Food Microbiol., 10, 175- 177.

## Highlights

- Primary models based on bacteria mechanistic behavior and heterogeneous population
- Novel inhibition differential equation to model co-culture of Lactic acid bacteria and *Listeria monocytogenes*
- Dynamic behavior analysis of heterogeneous population
- Experimental data fit and parameter effect analysis