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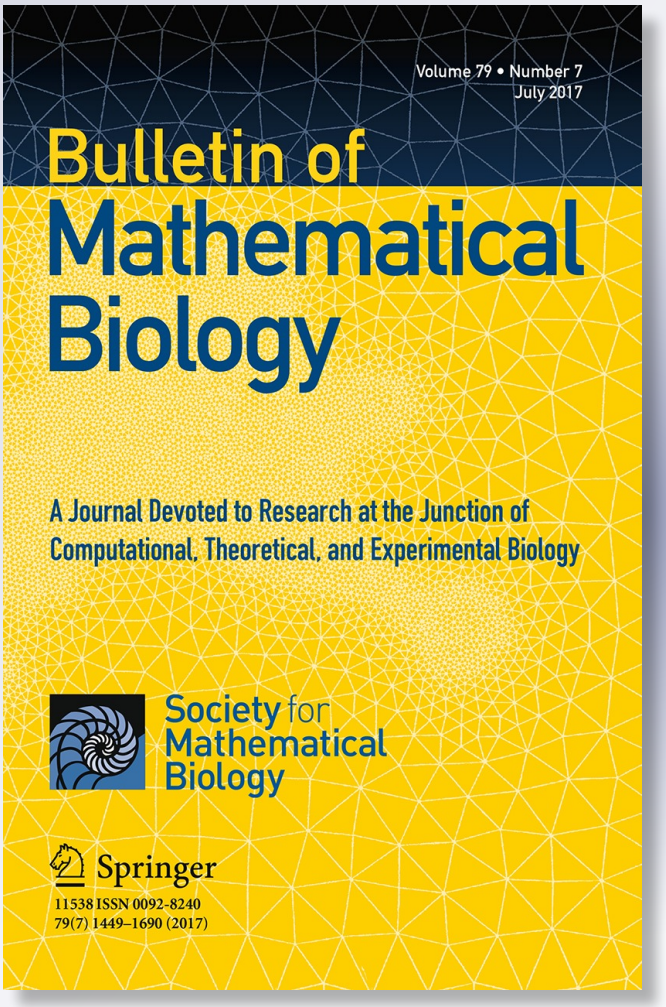

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ORIGINAL ARTICLE

Mathematical Model of Interaction Between Bacteriocin-Producing Lactic Acid Bacteria and Listeria. Part 1: Steady States and Thresholds

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Abstract Mathematical modeling is an important tool to assessing quantitative conjectures and to answer specific questions. In the modeling, we assume that a competitor represented by a lactic acid bacterium produces antimicrobial compounds (substances that kill microorganisms or inhibit their growth), such as lactic acid and bacteriocins, with some cost to its own growth. Bacteriocins are protein compounds with antimicrobial effect against related species and bacteria such as *Listeria monocytogenes*, which is foodborne pathogen that cause listeriosis. From the analysis of the model, we found the thresholds which determine the existence of multiple equilibria and we studied their stability, in order to evaluate the interaction between lactic acid bacteria and *L. monocytogenes*.

Keywords Mathematical model · Thresholds · Multiple equilibria · Bacteriocin · Stability analysis · Listeria

1 Introduction

Lactic acid bacteria (LAB) constitute a group of Gram-positive bacteria that have morphological, metabolic and physiological similarities, which produce lactic acid as

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the end product of carbohydrate metabolism (Khalid 2011). Gram-positive bacteria have a thick, relatively impermeable wall that resists decolorization and is composed of peptidoglycan and secondary polymers (Beveridge 2001).

LAB have been used for centuries in the fermentation of food for flavor and texture development, and also because of their ability to produce antimicrobial compounds which prevent the growth of spoilage and pathogenic microorganisms (De Vuyst and Vandamme 1994; Delves-Broughton et al. 1996). The antimicrobial activity of LAB is related to factors such as the decrease in pH levels, production of lactic acid, competition for substrates and production of substances with bactericidal action (lethal action, including bacteriocins) or bacteriostatic (which inhibit the growth). In the non-dissociated form, lactic acid passes through the cytoplasmic membrane of pH-sensitive bacteria, including *Listeria monocytogenes*, where the acid dissociates, acidifying the intracellular environment, the cell functions are inhibited, and the membrane potential (the difference in electric potential between the exterior and the interior of a biological cell) is annulled (Paparella et al. 2013).

Bacteriocins produced by some strains of LAB are ribosomally synthesized antimicrobial peptides or proteins and may offer a competitive advantage to them. The lethal action of the bacteriocin is a strategy to maintain the population of LAB by limiting the growth of competitor foodborne pathogens and guaranteeing greater access to nutrients and space. However, producer microorganisms are immune to their own bacteriocin (Cotter et al. 2005).

Apparently, bacteriocin production is stimulated by less favorable growth conditions, called stress factors, such as low temperatures, low specific growth rates and competing microflora. Slow growth can make more energy available required for both polymerization of building blocks (amino acids) and specific and coordinated control of bacteriocin gene expression (De Vuyst et al. 1996).

Cells in the initial phase of the growth curve are better suited for the enterocin production, a specific bacteriocin, and at later stages enterocin production drops to zero. Leroy and Vuyst (2002) concluded that the switch-off of this bacteriocin production could be related to cell density.

Listeria monocytogenes is an important foodborne pathogen due to its widespread distribution in nature (Juneja 2003), being able to tolerate high concentrations of salt and survive when exposed to a wide range of temperatures (1–45 °C) (Lewus and Montville 1991). It is found in food manufacturing plants, in the hands of manipulators and in the form of biofilm attached to the equipments (Moonchai et al. 2005). Listeria is difficult to be properly removed from equipment surfaces, which can survive for long periods and be responsible for outbreaks of listeriosis, which is caused by eating food contaminated with *Listeria monocytogenes* (Ferreira et al. 2014).

Foodborne listeriosis is a serious disease with high fatality rates (20–30%) compared to other foodborne pathogens. *Listeria monocytogenes* most often affects those with a severe underlying disease or condition (immunosuppression, HIV/AIDS, chronic conditions such as cirrhosis that impair the immune system); pregnant women; fetuses or neonates (WHO 2004; Altuntas et al. 2012). The clinical syndromes associated with listeriosis include mainly central nervous system infections and primary bacteremia, but can also include endocarditis. Meningitis, a possible manifestation of listeriosis, is seen mainly in the elderly and in immunocompromised patients (Farber

and Peterkin 1991). The control of this microorganism is a big challenge, especially in ready-to-eat foods which depend only on refrigeration for storage (Okada et al. 2013).

In last years, mathematical models to describe the growth of LAB (logistic, Gompertz equation or Monod equation) and the production of lactic acid and bacteriocin were developed. Due to complexity of the models, systems of ordinary differential equations were solved using, for example, Euler integration technique, and then, the models were validated using experimental results (Callewaert and Vuyst 2000; Charalampopoulos et al. 2009; Guerra et al. 2007; Liu et al. 2005; Luedeking and Piret 1959; Lv et al. 2005; Moonchai et al. 2005; Neysens et al. 2003; Pongtharangkul et al. 2008; Vázquez and Murado 2008). Due to numerical approach, these models have limited predictive value, especially when the conditions in which they were built are not satisfied.

Regulation of bacteriocin production, such as the nisin, is frequently mediated through two-component regulatory systems (sensor kinase and response regulator), often as part of a quorum sensing mechanism. The resulting nisin acts, besides the antimicrobial function, as a protein pheromone in regulating its own synthesis (Kleerebezem 2004; Riley 2009; Zhou et al. 2006). Quorum sensing refers to the ability of bacterial populations to coordinately regulate gene expression in response to changes in the local population density (Anguige et al. 2005; Ward et al. 2004). A mathematical model was developed to assess the effects of quorum sensing in the dynamic of *Lactococcus lactis* based on the regulatory system of the nisin biosynthesis (Delboni and Yang 2012). By analyzing the model, it was possible to verify this mechanism: when the nisin reaches a certain threshold concentration, the level of the phosphorylated response regulator increases significantly, showing that the regulatory system becomes activated.

In this work, we include quorum sensing in the growth rate of lactic acid bacteria and also in the rate of bacteriocin production. Considering that LAB also produce lactic acid that has antimicrobial action, we study the interaction between LAB and *Listeria monocytogenes*. The objective is to obtain the conditions for the existence and local stability of boundary equilibria in the domain of biological interest and the conditions for the existence of internal equilibrium points. The stability analysis yields the thresholds, values at which bifurcations occur.

This paper is structured as follows. In Sect. 2, a model describing the interaction between bacteriocin-producing lactic acid bacteria and Listeria is developed. In Sect. 3, the stability of the boundary equilibrium points is assessed, and some conditions for the existence of internal equilibria are presented. Discussions and biological interpretations are given in the same section, and conclusions are given in Sect. 4.

2 Mathematical Model

We present a mathematical model considering a homogeneous environment. We denote by $\hat{S}$ and $\hat{C}$ the populations of, respectively, LAB and Listeria in a given time $\hat{t}$. The concentrations of lactic acid and bacteriocin produced by LAB are represented by $\hat{A}$ and $\hat{B}$, respectively.

Firstly, we describe the effects of bacteriocin on the dynamics of LAB and Listeria. Logistic curve is appropriate to describe the growth of LAB, since it takes into account self-inhibition caused by the production of lactic acid and the depletion of nutrients (Leroy and Vuyst 2002). By assumption, the growth factor for LAB is multiplied by a function $\hat{F}(\hat{B})$, which mimics a cost for the bacteriocin production. This cost function is such that, when the concentration of bacteriocin is small, the LAB grow slowly, a feature that attempts to describe the adaptation to the environment, but, as the concentration increases, also increases the growth rate of bacteria. Sufficiently large quantity of bacteriocin demonstrates that bacteria are in sufficient number to establish in the environment, and they can reproduce without the bacteriocin to protect them from competitors. A function with these properties is given by

$$\hat{F}(\hat{B}) = 1 - \frac{\hat{\sigma}}{1 + \hat{\omega}\hat{B}},$$

where $\hat{\omega}$ is a positive parameter and $\hat{\sigma}$ represents the cost for bacteriocin production. Notice that $\hat{F}(0) = 1 - \hat{\sigma}$, then we must have $0 < \hat{\sigma} < 1$ because $\hat{\sigma} > 1$ would imply $\hat{F}(0) < 0$, which represents a negative growth rate.

The equation that describes the growth of LAB approaches the logistic equation when bacteria are better adapted. Then,

$$\frac{d\hat{S}}{d\hat{t}} = \hat{\phi}\left(1 - \frac{\hat{\sigma}}{1 + \hat{\omega}\hat{B}}\right)\left(1 - \frac{\hat{S}}{K_1}\right)\hat{S} - \hat{\mu_1}\hat{S},$$

where $\hat{\phi}$ is the intrinsic growth rate, K_1 is the carrying capacity and $\hat{\mu_1}$ is the mortality rate of LAB.

The bacteriocin production is influenced by the concentration of LAB; hence, the term corresponding to bacteriocin production is multiplied by a function $\hat{G}(\hat{S})$. From a metabolic point of view, bacteriocins are usually considered as primary metabolites, that is, products that are formed at a rate that depends only on the growth rate of the producing bacteria. However, few studies have considered LAB producing bacteriocins (secondary metabolites), such as pediocin AcH produced by *Pediococcus acidilactici* H, E, F and M, and propionicin produced by *Propionibacterium ihoenii* (Cabo et al. 2001). The production of primary and secondary metabolites has been described for the same species and peptide (De Vuyst et al. 1996).

The function $\hat{G}(\hat{S})$ must be such that, if the concentration of LAB is too small, the bacteriocin production rate is maximum, for instance, $\hat{G}(\hat{S}) \approx 1$. However, as the bacteria multiply and the population increases, they become more adapted to the environment. Consequently, the need for production of protective metabolites decreases, and $\hat{G}(\hat{S})$ becomes small. A function showing such behavior is given by

$$\hat{G}(\hat{S}) = 1 - \frac{\hat{\gamma}\hat{S}}{1 + \hat{\gamma}\hat{S}},$$

where $\hat{\gamma}$ is a positive parameter.

Luedeking and Piret (1959) concluded that the instantaneous rate of lactic acid formation is related to the instantaneous rate of LAB growth and the amount of bacteria. We also considered a degradation rate, proportional to the lactic acid concentration.

In the model, $\hat{\alpha_1}$ is the specific bacteriocin production rate related to the growth of LAB (primary), and $\hat{\alpha_2}$ is the number of molecules of bacteriocin produced by a lactic acid bacterium (secondary). Similarly, $\hat{\alpha_3}$ is the specific lactic acid production rate (primary) and $\hat{\alpha_4}$ is the number of molecules of bacteriocin produced by a lactic acid bacterium (secondary). The deactivation rates of these metabolites are represented by $\hat{\mu_2}$ for bacteriocin and $\hat{\mu_3}$ for the lactic acid. The activity of bacteriocin probably decreases due to the proteolytic degradation, aggregation or adsorption to cells (Leroy and Vuyst 2002).

Suppose that LAB and Listeria compete for the same limited nutrients. We consider an indirect competition model with antimicrobials produced by LAB, being lethal to the other species. The bacteriocin is produced with a certain cost to the LAB growth. Each species, in the absence of the other, has logistic growth (Listeria) or nearly logistic (LAB). Besides the logistic growth, we include the mortality terms $-\hat{\mu}_1\hat{S}$ and $-\hat{\mu}_4\hat{C}$.

The interaction between Listeria and the antimicrobial is directly proportional to this population and the metabolites involved in the interaction. It is assumed that the interaction is lethal for Listeria and decreases the metabolites concentration. The parameters $\hat{\delta_1}$ and $\hat{\delta_2}$ correspond to these interaction rates. The action of these metabolites produced by LAB occurs on the cytoplasmic membrane of Listeria. Thus, we assume that both lactic acid and the bacteriocin are "consumed" in the interaction with the Listeria, and they are reduced by $-\hat{\delta_1}\hat{A}\hat{C}$ and $-\hat{\delta_2}\hat{B}\hat{C}$, respectively, at each time. The parameter $1/\hat{\eta_1}$ is the number of lactic acid molecules binded to Listeria to disable it, as well as $1/\hat{\eta_2}$ is the number of bacteriocin molecules binded to Listeria to disable it. Thus, the Listeria population is reduced by $\hat{\eta_1}\hat{\delta_1}\hat{A}\hat{C}$ and $\hat{\eta_2}\hat{\delta_2}\hat{B}\hat{C}$ when the interaction occurs. The growth of Listeria population is limited by the carrying capacity K_4.

Based on above descriptions, the dynamic of the interaction between LAB and Listeria is described by the following system of non-linear ordinary differential equations

$$\begin{cases} \dfrac{d\hat{S}}{d\hat{t}} = \hat{\phi}\left(1-\dfrac{\hat{\sigma}}{1+\hat{\omega}\hat{B}}\right)\left(1-\dfrac{\hat{S}}{K_1}\right)\hat{S} - \hat{\mu_1}\hat{S} \\ \dfrac{d\hat{B}}{d\hat{t}} = \left(1-\dfrac{\hat{\gamma}\hat{S}}{1+\hat{\gamma}\hat{S}}\right)\left(\hat{\alpha_1}\dfrac{d\hat{S}}{d\hat{t}}+\hat{\alpha_2}\hat{S}\right) - \hat{\mu_2}\hat{B} - \hat{\delta_2}\hat{B}\hat{C} \\ \dfrac{d\hat{A}}{d\hat{t}} = \hat{\alpha_3}\dfrac{d\hat{S}}{d\hat{t}} + \hat{\alpha_4}\hat{S} - \hat{\mu_3}\hat{A} - \hat{\delta_1}\hat{A}\hat{C} \\ \dfrac{d\hat{C}}{d\hat{t}} = \hat{\phi}\left(1-\dfrac{\hat{C}}{K_4}\right)\hat{C} - \hat{\mu_4}\hat{C} - \hat{\eta_1}\hat{\delta_1}\hat{A}\hat{C} - \hat{\eta_2}\hat{\delta_2}\hat{B}\hat{C}. \end{cases} \tag{1}$$

Table 1 Dimensionless variables and meanings (2)

Variable	Transformation	Meaning
S	$\hat{S}/K_1$	Proportion of population of LAB
B	$\hat{B}\hat{\omega}$	Relative concentration of bacteriocin
A	$\hat{A}\hat{\phi}/K_1$	Relative concentration of lactic acid
C	$\hat{C}/K_4$	Proportion of population of Listeria

Table 2 Dimensionless parameters and meanings (2)

Parameter	Transformation	Meaning
μ_1	$\hat{\mu_1}/\hat{\phi}$	Relative mortality of LAB
μ_4	$\hat{\mu_4}/\hat{\phi}$	Relative mortality of Listeria
α_1	$\hat{\alpha_1}\hat{\omega}K_1$	Relative production of bacteriocin related to the growth of LAB
α_2	$\hat{\alpha_2}\hat{\omega}K_1/\hat{\phi}$	Relative number of bacteriocin molecules produced by LAB
α_3	$\hat{\alpha_3}\hat{\phi}$	Relative production of lactic acid related to the growth of LAB
α_4	$\hat{\alpha_4}$	Relative number of lactic acid molecules produced by LAB
δ_1	$\hat{\delta_1}K_4/\hat{\phi}$	Relative number of interactions between lactic acid and Listeria
δ_2	$\hat{\delta_2}K_4/\hat{\phi}$	Relative number of interactions between bacteriocin and Listeria
$1/\eta_1$	$\phi K_4/\hat{\eta_1}K_1$	Relative number of lactic acid molecules binded to Listeria to disable it
$1/\eta_2$	$\phi K_4\hat{\omega}/\hat{\eta_2}$	Relative number of bacteriocin molecules binded to Listeria to disable it
μ_2	$\hat{\mu_2}/\hat{\phi}$	Relative deactivation of bacteriocin
μ_3	$\hat{\mu_3}/\hat{\phi}$	Relative deactivation of lactic acid
σ	$\hat{\sigma}$	Cost for bacteriocin production

We apply a suitable transformation of variables of the model (1) to deal with dimensionless system of equations. The non-dimensional variables and parameters are defined in Tables 1 and 2. Hence, dimensionless dynamics of the interaction between bacteriocin-producing lactic acid bacteria and Listeria is described by the following system of equations

$$\begin{cases} \dfrac{dS}{dt} = \left(1 - \dfrac{\sigma}{1+B}\right)(1-S)S - \mu_1 S \\ \dfrac{dB}{dt} = \left(1 - \dfrac{\gamma S}{1+\gamma S}\right)\left(\alpha_1 \dfrac{dS}{dt} + \alpha_2 S\right) - \mu_2 B - \delta_2 BC \\ \dfrac{dA}{dt} = \alpha_3 \dfrac{dS}{dt} + \alpha_4 S - \mu_3 A - \delta_1 AC \\ \dfrac{dC}{dt} = (1-C)C - \mu_4 C - \eta_1\delta_1 AC - \eta_2\delta_2 BC. \end{cases} \tag{2}$$

Proposition 1 (Positively invariant domain) *The set:*

$$\Omega = \left\{(S, B, A, C) \in \mathbb{R}^4 \,/\, 0 \leq S \leq 1, 0 \leq B \leq B^{th}, 0 \leq A \leq A^{th}, 0 \leq C \leq 1\right\},$$

such that: $0 < \sigma < 1$, $\alpha_2 > \alpha_1\mu_1$ *and* $\alpha_4 > \alpha_3\mu_1$, *where*

$$B^{th} = \frac{\alpha_1 + \alpha_2}{\mu_2} \quad \text{and} \quad A^{th} = \frac{\alpha_3 + \alpha_4}{\mu_3}$$

represents the region of biological interest, where all variables are non-negative. This domain is positively invariant under the induced flow by the system (2).

In fact by analyzing the vector field, all points in Ω remain within this region or in the boundary (for details see Delboni (2015)).

The hypotheses $\alpha_2 > \alpha_1\mu_1$ and $\alpha_4 > \alpha_3\mu_1$ are necessary to the domain Ω be positively invariant. Then, we are considering that these conditions are being always satisfied hereafter, except when explicitly cited.

3 Analysis of the Model

The equations in the steady state are obtained letting the time derivatives in the system (2) equal to zero. We obtain the equilibrium points denoted by $(\bar{S}, \bar{B}, \bar{A}, \bar{C})$, but it is necessary to determine the conditions under which these equilibria are biologically feasible (i.e., non-negative values).

In this section, we will present the conditions for the existence and stability of trivial, and boundary equilibria, and the existence of internal equilibrium points, which depend on myriad of conditions.

The local stability of the equilibrium points is determined by the eigenvalues of the characteristic equation $\boldsymbol{\Psi}(\lambda) = \det(\mathbf{J} - \lambda\mathbf{I}) = 0$, where $\mathbf{J}$ is Jacobian evaluated at the equilibrium point under analysis.

3.1 Trivial Equilibrium

It corresponds to the equilibrium without microorganisms and metabolites, $\mathbf{E_0} = (0, 0, 0, 0)$. It always exists and, if $\sigma_1 = 1 - \mu_1 < 0$ and $\sigma_4 = 1 - \mu_4 < 0$, is the only equilibrium biologically feasible, i.e., with non-negative coordinates. The interpretation of the condition $\sigma_4 < 0$ is obvious, since the threshold is the difference between intrinsic growth rate and mortality for Listeria. If the net reproduction rate σ_4 is negative, Listeria is extinguished. Similarly, it is concluded that for $\sigma_1 < 0$, the LAB cannot be established in the environment.

The trivial equilibrium represents the extinction of both bacteria, and obviously, the absence of the antimicrobial compounds (lactic acid and bacteriocin) that depend on lactic acid bacteria to produce them.

Theorem 1 (Local stability of $\mathbf{E_0}$) *If* $\sigma_4 < 0$ *and* $\sigma > \sigma_1$, $\mathbf{E_0}$ *is locally asymptotically stable in* Ω. *If* $\sigma < \sigma_1$ *and/or* $\sigma_4 > 0$, *the point* $\mathbf{E_0}$ *is unstable in* Ω.

Proof Analyzing the eigenvalues $\lambda_1 = \sigma_1 - \sigma$, $\lambda_2 = -\mu_2$, $\lambda_3 = -\mu_3$ and $\lambda_4 = \sigma_4$, we conclude this theorem. □

As expected, if the growth conditions for these bacteria are inadequate, the point E_0 is locally asymptotically stable, i.e., Listeria and LAB become extinct.

3.2 Boundary Equilibria

3.2.1 Only Listeria

This corresponds to the equilibrium $\mathbf{E_c} = (0, 0, 0, \sigma_4)$, which is biologically feasible if $\sigma_4 > 0$. In order to the population of Listeria to prevail, there must be good growth condition for them ($\sigma_4 > 0$), and inadequate condition for lactic bacteria ($\sigma > \sigma_1$), as we shall see in the following theorem.

Theorem 2 (Local stability of $\mathbf{E_c}$) *If $\sigma_4 > 0$ and $\sigma > \sigma_1$, the point $\mathbf{E_c} = (0, 0, 0, \sigma_4)$ is locally asymptotically stable in Ω. If $\sigma < \sigma_1$, it is unstable.*

Proof We concluded by analyzing the eigenvalues $\lambda_1 = \sigma_1 - \sigma$, $\lambda_2 = -(\mu_2 + \delta_2\sigma_4)$, $\lambda_3 = -(\mu_3 + \delta_1\sigma_4)$ and $\lambda_4 = -\sigma_4$. □

3.2.2 Only Lactic Acid Bacterium

This corresponds to equilibrium $\mathbf{E_s} = (S_s, B_s, A_s, 0)$. Assuming that $\sigma_1 > 0$, the non-null coordinates are given by

$$S_s = \frac{\sigma_1 - \sigma + \sigma_1 B_s}{1 - \sigma + B_s}, \quad A_s = \frac{\alpha_4(\sigma_1 - \sigma + \sigma_1 B_s)}{\mu_3(1 - \sigma + B_s)} \tag{3}$$

and B_s is the positive solution of

$$h_1(B) = c_{21}B^2 + c_{11}B + c_{01} = 0, \tag{4}$$

where

$$\begin{cases} c_{21} = -\mu_2(1 + \sigma_1\gamma) \\ c_{11} = -\mu_2[(1 - \sigma) + \gamma(\sigma_1 - \sigma)] + \alpha_2\sigma_1 \\ c_{01} = \alpha_2(\sigma_1 - \sigma). \end{cases} \tag{5}$$

In order to discuss thresholds and critical parameters, we divide the discussion in two cases: $\sigma < \sigma_1$ (low cost for bacteriocin production) and $\sigma > \sigma_1$ (high cost for bacteriocin production).

Remark 1 After analyzing $h_1(B)$, it is concluded that:

(i) if $\sigma < \sigma_1$, there is an equilibrium point $\mathbf{E_s} = (S_s, B_{22}, A_s, 0)$, where B_{22} is the only positive solution of $h_1(B) = 0$;

(ii) for $\sigma \in (\sigma_1, 1)$, if $\alpha_2 > \alpha_2^+(\sigma)$, where α_2^+ is the greater solution that annuls the discriminant $\Delta(\alpha_2) = c_{11}^2 - 4c_{21}c_{01}$, then $h_1(B)$ has two positive roots, with $B_{12} < B_{22}$, and there are two equilibria $\mathbf{E_s}$, denoted by $\mathbf{E_s^+} = (S_s^+, B_{22}, A_s^+, 0)$ and $\mathbf{E_s^-} = (S_s^-, B_{12}, A_s^-, 0)$, where

$$\alpha_2^+ = \frac{2\mu_2[(1+\sigma_1\gamma)(\sigma-\sigma_1)+\sigma(1-\sigma_1)] + \sqrt{\tilde{\Delta}}}{2\sigma_1^2}, \tag{6}$$

and $\tilde{\Delta} = 16\sigma(1-\sigma_1)(1+\sigma_1\gamma)(\sigma-\sigma_1)\mu_2^2 > 0$.

(iii) if $\sigma > \sigma_1$, but $\alpha_2 < \alpha_2^+$, there is not positive equilibrium $\mathbf{E_s}$.

For successful use of LAB in food biopreservation, we must assess the conditions that enable the elimination of foodborne pathogens, such as Listeria. Mathematically speaking, we need to determine the conditions for the stability of the equilibrium without Listeria ($\mathbf{E_s}$). Such conditions are provided in Theorem 3 (for $\sigma < \sigma_1$) and Theorem 4 (for $\sigma > \sigma_1$). However, it is important to highlight that, in these theorems, we guaranteed only the local stability of the equilibrium point $\mathbf{E_s}$. If there are conditions for the existence of multiple internal equilibria (see Sect. 3.3), the extinction of Listeria depends also on the initial contamination, since some internal equilibrium $\mathbf{E_*}$ can be locally asymptotically stable.

Detailed analysis of cases (i) and (ii) are presented.

In the next theorem, we consider case (i), that is, low cost for the bacteriocin production ($0 < \sigma < \sigma_1$).

Theorem 3 (Local stability of $\mathbf{E_s}$ **for** $\sigma < \sigma_1$) *Supposing that* $\sigma < \sigma_1$ *and* $\sigma_4 < 0$, *the unique equilibrium point without Listeria,* $\mathbf{E_s} = (S_s, B_s, A_s, 0)$, *is locally asymptotically stable in* Ω. *If* $\sigma_4 > 0$, *and one of the following additional conditions to the parameters* α_2 *and/or* α_4 *is satisfied:*

(i) $\alpha_2 \geq \max\{\alpha_1\mu_1, \alpha_{24}\}$, *or*
(ii) $\alpha_1\mu_1 < \alpha_{24}$, $\alpha_1\mu_1 < \alpha_2 < \alpha_{24}$ *and* $\alpha_4 > \max\{\alpha_3\mu_1, \alpha_{41}\}$, *or*
(iii) $\alpha_2 > \alpha_1\mu_1$ *and* $\alpha_4 \to +\infty$,

then $\mathbf{E_s}$ *is locally asymptotically stable in* Ω. *However, if* $\alpha_1\mu_1 < \alpha_{24}$, $\alpha_1\mu_1 < \alpha_2 < \alpha_{24}$, $\alpha_3\mu_1 < \alpha_{41}$ *and* $\alpha_3\mu_1 < \alpha_4 < \alpha_{41}$, *then* $\mathbf{E_s}$ *is unstable, where:*

$$\alpha_{24} = \frac{\sigma_4\mu_2\{\sigma_4(1+\sigma_1\gamma)+\eta_2\delta_2[(1-\sigma)-\gamma(\sigma-\sigma_1)]\}}{\eta_2\delta_2[\sigma_4\sigma_1+\eta_2\delta_2(\sigma_1-\sigma)]}, \tag{7}$$

$$\alpha_{41} = \frac{\mu_3\eta_2\delta_2(B_{11}-B_{22})(1-\sigma+B_{22})}{\eta_1\delta_1(\sigma_1-\sigma+\sigma_1B_{22})} \quad \text{and} \quad B_{11} = \frac{\sigma_4}{\eta_2\delta_2}. \tag{8}$$

Proof If $\alpha_2 < \alpha_{24}$, it is possible to show that $B_{11} > B_{22}$ and, then, $\alpha_{41} > 0$ (for details and demonstration see Delboni (2015)).

Evaluating the Jacobian $\mathbf{J}$ in $\mathbf{E_s} = (S_s, B_s, A_s, 0)$, we obtain

$$\mathbf{J_s} = \mathbf{J}(S_s, B_s, A_s, 0) = \begin{bmatrix} -\left(1 - \dfrac{\sigma}{1+B_s}\right) S_s & \dfrac{\sigma(1-S_s)S_s}{(1+B_s)^2} & 0 & 0 \\ J_{21} & J_{22} & 0 & -\delta_2 B_s \\ J_{31} & J_{32} & -\mu_3 & -\delta_1 A_s \\ 0 & 0 & 0 & J_{44} \end{bmatrix},$$

where:

$$\begin{cases} J_{21} = \dfrac{-\gamma\alpha_2 S_s}{(1+\gamma S_s)^2} + \left(\dfrac{1}{1+\gamma S_s}\right)\left[\alpha_2 - \alpha_1 S_s\left(1 - \dfrac{\sigma}{1+B_s}\right)\right] \\ J_{22} = \left(\dfrac{1}{1+\gamma S_s}\right)\left[\dfrac{\sigma}{(1+B_s)^2}(1-S_s)\right]\alpha_1 S_s - \mu_2 \\ J_{31} = -\alpha_3 S_s\left(1 - \dfrac{\sigma}{1+B_s}\right) + \alpha_4 \\ J_{32} = \dfrac{\alpha_3\sigma}{(1+B_s)^2}(1-S_s)S_s \\ J_{44} = \sigma_4 - \eta_1\delta_1 A_s - \eta_2\delta_2 B_s. \end{cases}$$

The eigenvalues are determined by the characteristic equation

$$\boldsymbol{\Psi}(\lambda) = (-\mu_3 - \lambda) \times \boldsymbol{\Psi}_1(\lambda) \times \boldsymbol{\Psi}_2(\lambda) \;=\; 0,$$

where

$$\boldsymbol{\Psi}_1(\lambda) = (\sigma_4 - \eta_1\delta_1 A_s - \eta_2\delta_2 B_s) - \lambda \quad \text{and} \quad \boldsymbol{\Psi}_2(\lambda) = \lambda^2 + a_1\lambda + a_0,$$

with a_1 and a_0 being given by

$$a_1 = \left(\frac{1-\sigma+B_s}{1+B_s}\right) S_s + \frac{\alpha_1 S_s(1-S_s)[(1-\sigma)(1+2B_s) + B_s^2]}{B_s(1+\gamma S_s)(1+B_s)^2} + \frac{S_s(\alpha_2 - \alpha_1\mu_1)}{B_s(1+\gamma S_s)},$$

and

$$a_0 = \frac{\mu_2[(\sigma_1 - \sigma) + B_s S_s]}{1+B_s} + \frac{\alpha_2\gamma S_s^2(\sigma_1 - S_s)}{(1+B_s)(1+\gamma S_s)^2}.$$

One eigenvalue is $\lambda_1 = -\mu_3 < 0$. The coefficient a_1, considering that $\sigma < 1$, $0 < S_s < 1$ and $\alpha_2 > \alpha_1\mu_1$, is positive. The coefficient a_0 is positive, considering $B_s > 0$, $S_s < \sigma_1$ and $\sigma < \sigma_1$. Thus, using the Routh–Hurwitz criteria (Murray 2001), the eigenvalues λ_3 and λ_4 are negative or have negative real part (if complex). If $\sigma_4 \leq 0$, then $\lambda_2 < 0$. If $\sigma_4 > 0$, substituting A_s in Eq. (3) in λ_2, we obtain:

$$\lambda_2 = \sigma_4 - \eta_1\delta_1 A_s - \eta_2\delta_2 B_s = \frac{\eta_1\delta_1(\sigma_1 - \sigma + \sigma_1 B_s)(\alpha_{41} - \alpha_4)}{\mu_3(1 - \sigma + B_s)}.$$

For $\sigma < \sigma_1$, and knowing that under above conditions the only positive solution of $h_1(B) = 0$ is $B_s = B_{22}$, we prove this theorem. □

Theorem 3 deals with low cost for bacteriocin production by LAB and that LAB are in an adequate environment, with favorable conditions for growth and bacteriocin production, as pH and temperature. Listeria cannot survive if $\sigma_4 < 0$, and the unique locally asymptotically stable equilibrium is $\mathbf{E_s} = (S_s, B_s, A_s, 0)$. When $\sigma_4 > 0$, there are good conditions for the proliferation of Listeria. However, as we have proved, if the bacteriocin production is high enough ($\alpha_2 \geq \max\{\alpha_1\mu_1, \alpha_{24}\}$, with α_{24} given by expression (7)), the equilibrium $\mathbf{E_s}$ is locally asymptotically stable. With low bacteriocin production ($\alpha_1\mu_1 < \alpha_2 < \alpha_{24}$), but high lactic acid production ($\alpha_4 > \max\{\alpha_3\mu_1, \alpha_{41}\}$, with α_{41} given by expression (8)), it is also possible to guarantee the local stability of the equilibrium without Listeria.

Let us consider case (ii). With high cost for bacteriocin production ($\sigma > \sigma_1$), as we presented in Remark 1, if $\alpha_2 > \max\left\{\alpha_2^+, \alpha_1\mu_1\right\}$ (with α_2^+ given by (6)) there are two equilibrium points without Listeria. One of them with low bacteriocin concentration ($\mathbf{E_s^-} = (S_s^-, B_{12}, A_s^-, 0)$) and another with higher concentration ($\mathbf{E_s^+} = (S_s^+, B_{22}, A_s^+, 0)$). This means that a satisfactory production of bacteriocin is required, since it also interferes with the growth of LAB. In Theorem 4, threshold conditions are presented for the parameters α_2 and α_4, just as in Theorem 3, and also conditions for the parameter δ_2, which indicates the interaction between bacteriocin and Listeria. We will prove that, if the bacteriocin activity is high, i.e., if the interaction rate between bacteriocin and Listeria is high ($\delta_2 > \delta_{2d}$), it does not matter the productivity of lactic acid and bacteriocin, provided, of course, $\alpha_2 > \max\left\{\alpha_2^+, \alpha_1\mu_1\right\}$. The threshold δ_{2d} is given by

$$\delta_{2d} = \frac{\sigma_4\sigma_1(1+\sigma_1\gamma)}{\eta_2(\sigma-\sigma_1)(1+\sigma_1\gamma)+\eta_2\sqrt{\Delta_3}},\quad \Delta_3 = \sigma(1-\sigma_1)(\sigma-\sigma_1)(1+\sigma_1\gamma), \tag{9}$$

which is obtained by comparing thresholds for α_2, which come from α_2^+ and α_{24} (details and demonstrations can be seen in Delboni (2015)). With low bacteriocin activity ($\delta_2 < \delta_{2d}$), high production of lactic acid ($\alpha_4 > \max\{\alpha_3\mu_1, \alpha_{41}\}$), or high production of bacteriocin ($\alpha_2 > \alpha_{24}$), is required as an additional condition to ensure the local stability of $\mathbf{E_s^+}$.

Theorem 4 (Local stability of $\mathbf{E_s}$ **for** $\sigma > \sigma_1$) *Suppose that* $\sigma > \sigma_1$ *and* $\alpha_2 > \max\left\{\alpha_2^+, \alpha_1\mu_1\right\}$, *with* α_2^+ *given by* (6). *Then, there are two equilibrium points without Listeria:* $\mathbf{E_s^+} = (S_s^+, B_{22}, A_s^+, 0)$ *and* $\mathbf{E_s^-} = (S_s^-, B_{12}, A_s^-, 0)$. *The point* $\mathbf{E_s^-}$ *is always unstable. If* $\sigma_4 < 0$, *then* $\mathbf{E_s^+}$ *is locally asymptotically stable in* Ω. *For* $\sigma_4 > 0$, *considering the threshold for* δ_2 *in* (9), *and if one of the following additional conditions to the parameters* δ_2, α_2 *and/or* α_4 *is satisfied:*

(i) $\delta_2 < \delta_{2d}$, $\max\left\{\alpha_1\mu_1, \alpha_2^+\right\} < \alpha_2 < \alpha_{24}$ *and* $\alpha_4 > \max\{\alpha_3\mu_1, \alpha_{41}\}$, *or*
(ii) $\delta_2 < \delta_{2d}$, $\alpha_2 > \alpha_{24}$ *and* $\alpha_4 > \alpha_3\mu_1$, *or*
(iii) $\delta_2 > \delta_{2d}$, $\alpha_2 > \max\left\{\alpha_1\mu_1, \alpha_2^+\right\}$ *and* $\alpha_4 > \alpha_3\mu_1$,

then $\mathbf{E_s^+}$ *is locally asymptotically stable in* Ω. *But if*

$$\delta_2 < \delta_{2d},\quad \max\left\{\alpha_1\mu_1, \alpha_2^+\right\} < \alpha_2 < \alpha_{24} \quad \textit{and} \quad \alpha_3\mu_1 < \alpha_4 < \alpha_{41},$$

then $\mathbf{E_s^+}$ *is unstable.*

Proof The conditions for the existence of the points $\mathbf{E_s^-}$ and $\mathbf{E_s^+}$ have already been discussed in Remark 1. The Jacobian matrix was determined in Theorem 3. From the equation $\boldsymbol{\Psi}_2(\lambda) = 0$, by analyzing the coefficient a_1, it follows that, if $0 < \sigma < 1$, $S_s < 1$ and $\alpha_2 > \alpha_1\mu_1$, then $a_1 > 0$ for both B_{12} and B_{22}. In order to apply Routh–Hurwitz criteria, the signal of a_0 must be evaluated. Calculating the coefficient $a_0(B)$ and replacing the equilibrium condition $C = 0$ and $S = S_s$, we obtain an expression in terms of B given by

$$a_0(B) = \frac{\sigma_1(B - B_{13}) \times q(B)}{(1 + B)[(1 + B)(1 + \gamma\sigma_1) - \sigma(1 + \gamma)]^2}, \tag{10}$$

where

$$q(B) = -\alpha_2\sigma(1 - \sigma_1) + \mu_2[(1 + B)(1 + \gamma\sigma_1) - \sigma(1 + \gamma)]^2. \tag{11}$$

Supposing $\alpha_2 > \max\{\alpha_1\mu_1, \alpha_2^+\}$, there are two equilibrium points containing only LAB: $\mathbf{E_s^+} = (S_s^+, B_{22}, A_s^+, 0)$ and $\mathbf{E_s^-} = (S_s^-, B_{12}, A_s^-, 0)$, where B_{12} and B_{22} are the solutions of $h_1(B) = 0$ such that $0 < B_{13} < B_{12} < B_{22}$. Thus, to study the signal of a_0 given by (10), it is enough to study the signal of $q(B)$ given by (11), because other terms are positive. Calculating $q(B_{22})$ and considering

$$\begin{aligned}\Delta(\alpha_2) = {} & \alpha_2^2\sigma_1^2 + \mu_2^2[(1 - \sigma) - \gamma(\sigma - \sigma_1)]^2 \\ & -2\alpha_2\mu_2[(\sigma - \sigma_1)(1 + \gamma\sigma_1) + \sigma(1 - \sigma_1)],\end{aligned}$$

we have:

$$q(B_{22}) = \frac{\Delta(\alpha_2) + (\alpha_2\sigma_1 + \mu_2 k_1)\sqrt{\Delta(\alpha_2)}}{2\mu_2},$$

where $k_1 = [(1 - \sigma) - \gamma(\sigma - \sigma_1)]$. Supposing $\sigma > \sigma_1$ and $\alpha_2 > \alpha_2^+$, it follows that $\Delta(\alpha_2) > 0$. If $k_1 > 0$, we can see that $q(B_{22}) > 0$ and therefore $a_0(B_{22}) > 0$. When $k_1 < 0$, we observe that if $\alpha_2 > -\mu_2 k_1/\sigma_1$, then $q(B_{22}) > 0$. Calculating $\Delta(-\mu_2 k_1/\sigma_1)$, we have $\alpha_2^+ > -\mu_2 k_1/\sigma_1$. Considering that the function $\Delta(\alpha_2)$ is represented by a second-degree polynomial with roots α_2^- and α_2^+, and $\Delta(-\mu_2 k_1/\sigma_1) < 0$, it follows that $\alpha_2^- < \frac{-\mu_2 k_1}{\sigma_1} < \alpha_2^+$. With the hypothesis $\alpha_2 > \alpha_2^+$, we conclude that $q(B_{22}) > 0$. Therefore $a_0(B_{22}) > 0$ for $\sigma > \sigma_1$ and $\alpha_2 > \alpha_2^+$. Calculating $q(B_{12})$, we have

$$q(B_{12}) = \frac{\Delta(\alpha_2) - (\alpha_2\sigma_1 + \mu_2 k_1)\sqrt{\Delta(\alpha_2)}}{2\mu_2} < 0.$$

It follows that $a_0(B_{12}) < 0$ and $\mathbf{E_s^-} = (S_s^-, B_{12}, A_s^-, 0)$ is unstable.

For the equilibrium $\mathbf{E_s^+} = (S_s^+, B_{22}, A_s^+, 0)$, we conclude that $a_1(B_{22}) > 0$ and $a_0(B_{22}) > 0$, and therefore, λ_3 and λ_4 are negative, or have negative real part if complex. If $\sigma_4 \leq 0$, then $\lambda_2 = \sigma_4 - \eta_1\delta_1 A_s - \eta_2\delta_2 B_{22} < 0$, when we replace Eq. (3) in λ_2, as done in Theorem 3, considering $\sigma > \sigma_1$ and knowing that $B_{22} > B_{13}$, $A_s > 0$ and $B_{22} > 0$. □

3.3 Internal Equilibria

The coordinates of the equilibrium $\mathbf{E}_* = (S_*, B_*, A_*, C_*)$, where LAB and Listeria coexist (all positive variables), are:

$$S_* = \frac{\sigma_1 - \sigma + \sigma_1 B_*}{1 - \sigma + B_*},\ C_* = \frac{c_{21}B_*^2 + c_{11}B_* + c_{01}}{c_{22}B_*^2 + c_{12}B_*},\ A_* = \frac{\alpha_4 S_*}{\mu_3 + \delta_1 C_*},$$

where c_{21}, c_{11} and c_{01} are given by (4), and

$$\begin{cases} c_{22} = \delta_2(1 + \sigma_1\gamma) \\ c_{12} = \delta_2[(1 - \sigma) + \gamma(\sigma_1 - \sigma)]. \end{cases} \tag{12}$$

The coordinate B_* is the solution of the equation

$$h(B) = f(B) - g(B) = 0, \quad \text{with} \quad f(B) = [f_1(B) - h_1(B)] \times f_2(B). \tag{13}$$

The functions $f_1(B)$, $f_2(B)$ and $g(B)$ are

$$\begin{cases} f_1(B) = \eta_2\delta_2(B_{11} - B) \times (c_{22}B + c_{12}) \times B = \eta_2\delta_2(B_{11} - B) \times h_5(B) \times B \\ f_2(B) = (B - B_{41}) \times (d_2B^2 + d_1B + d_0) = (B - B_{41}) \times h_2(B) \\ g(B) \ = \eta_1\delta_1\alpha_4 \times \sigma_1(B - B_{13}) \times [h_5(B)]^2 \times B^2, \end{cases}$$

$h_1(B)$ is given by Eq. (4), with the coefficients $c'_{ij}s$ being defined in (5), $h_5(B) = c_{22}B + c_{12}$, with coefficients given by (12), and

$$h_2(B) = d_2B^2 + d_1B + d_0, \tag{14}$$

with coefficients given by

$$\begin{cases} d_2 = (\mu_3\delta_2 - \mu_2\delta_1)(1 + \sigma_1\gamma) \\ d_1 = (\mu_3\delta_2 - \mu_2\delta_1)[(1 - \sigma) + \gamma(\sigma_1 - \sigma)] + \delta_1\alpha_2\sigma_1 \\ d_0 = \delta_1\alpha_2(\sigma_1 - \sigma). \end{cases}$$

The roots of Eq. (13) are

$$\begin{cases} B_{i2},\ i = 1, 2, \text{ are solutions of } h_1(B_{i2}) = 0, \\ B_{j1},\ j = 5, 6, \text{ are solutions of } h_2(B_{j1}) = 0, \\ f_1(B) = 0 \ \Rightarrow \ B = B_{11} = \dfrac{\sigma_4}{\eta_2\delta_2}, \text{ and } B = B_{21} = -\dfrac{c_{12}}{c_{22}}, \\ f_2(B) = 0 \ \Rightarrow \ B = B_{41} = -(1 - \sigma), \text{ and } h_2(B) = 0, \\ g(B)=0 \ \Rightarrow \ B=B_{13}=\dfrac{(\sigma-\sigma_1)}{\sigma_1}, \ B=B_{21}=-\dfrac{c_{12}}{c_{22}} \text{ (multiplicity 2), and } B=0. \end{cases} \tag{15}$$

Remark 2 We consider two cases:

(i) $\sigma \in (0, \sigma_1)$. Denoting B_{22} as the positive root of $h_1(B) = 0$, if $0 < B < B_{22}$, then the coordinates of the internal equilibrium satisfy $S_* > 0$, $C_* > 0$ and $A_* > 0$. With respect to the equation $h_2(B) = 0$, by analyzing the signs of the coefficients d_2, d_1 and d_0, we verified that: (a) if $\delta_2/\mu_2 < \delta_1/\mu_3$, then $B_{51} < 0$ and $B_{61} > 0$, and (b) if $\delta_2/\mu_2 > \delta_1/\mu_3$, then $B_{51} < 0$ and $B_{61} < 0$, because $\Delta_2 = d_1^2 - 4d_2d_0 > 0$.

(ii) $\sigma \in (\sigma_1, 1)$. In order to obtain $S_* > 0$, $C_* > 0$ and $A_* > 0$, it is necessary $\alpha_2 > \alpha_2^+$ and $B_* > 0$, such that $B_{21} < B_{13} < B_{12} < B_* < B_{22}$. With respect to the equation $h_2(B) = 0$: (a) if $\delta_2/\mu_2 > \delta_1/\mu_3$, then $B_{51} < 0$ and $B_{61} > 0$, and (b) if $\delta_2/\mu_2 < \delta_1/\mu_3$, we conclude that $h_2(B) = 0$ has two positive solutions with $B_{51} < B_{61}$.

Corollary 1 follows from Remark 2:

Corollary 1 (Absence of feasible solutions) *If $\sigma_1 < 0$ and $\sigma_4 < 0$, there is not positive internal equilibrium for the system (2). There is not biologically feasible solution if $\sigma_1 > 0$, $\sigma_4 > 0$, but $\alpha_2 < \alpha_2^+$, α_2^+ given by (6), and $\sigma_1 < \sigma < 1$.*

If the bacteriocin production rate is small ($\alpha_2 < \alpha_2^+$), Corollary 1 states that there is not the equilibrium $\mathbf{E_*}$. This fact arises due to the variation of the bacteriocin concentration at time, dB/dt, which becomes negative in these conditions. In fact, using $S > 0$ in equilibrium from $dS/dt = 0$ and replacing it in the dynamic equation for B, we get:

$$\frac{dB}{dt} = \frac{h_1(B)}{(1+\gamma S)(1-\sigma+B)} - \delta_2 BC < 0, \quad \text{for} \quad \alpha_2 < \alpha_2^+.$$

The discriminant $\Delta(\alpha_2)$ of $h_1(B) = 0$ has positive roots $\alpha_2^- < \alpha_2^+$. For $0 < \alpha_2 < \alpha_2^-$, the discriminant is positive, but $h_1(B)$ has only negative solutions. For $\alpha_2^- < \alpha_2 < \alpha_2^+$, we have $\Delta(\alpha_2) < 0$, and $h_1(B) = 0$ does not have real solutions. Anyway, we can check that if $B > 0$, then $h_1(B) < 0$.

After the study of the functions $f_4(B) = f_1(B) - h_1(B)$ and $f(B)$ for $\sigma < \sigma_1$ (Delboni 2015), we summarize results related to two cases in next two sections.

3.3.1 $\sigma < \sigma_1$. Viable Solutions Are Such that $0 < B < B_{22}$.

The relative position of the roots of $h(B)$, given by Eq. (13) for $\sigma < \sigma_1$, is such that $B_{41} < B_{21} < B_{12} < B_{13} < 0 < B_{22}$, and the position of B_{61} depends on the signal of $(\mu_3\delta_2 - \mu_2\delta_1)$, as discussed in Remark 2. In Table 3, we present the sign of the function $h(B)$ in each intervals constructed with these roots.

Using intermediate value theorem, and taking into account the signals in Table 3, we conclude that there is at least one root to the equation $h(B) = 0$ in following intervals: (i) if $(\mu_3\delta_2 - \mu_2\delta_1) < 0$, then in (B_{41}, B_{21}), (B_{21}, B_{12}), (B_{12}, B_{13}) and $(B_{61}, +\infty)$, and (ii) if $(\mu_3\delta_2 - \mu_2\delta_1) > 0$, then in $(-\infty, B_{41})$, (B_{41}, B_{21}), (B_{21}, B_{12}) and $(B_{61}, 0)$. Therefore, when $\sigma < \sigma_1$ and regardless of the signal of $(\mu_3\delta_2 - \mu_2\delta_1)$, there are at most two viable solutions.

Proposition 2 with the hypothesis $B_{22} < B_{11}$ ($\alpha_2 < \alpha_{24}$) is stated.

Table 3 Signal for the function $h(B)$ considering $\sigma < \sigma_1$, (i) $(\mu_3\delta_2 - \mu_2\delta_1) < 0$ and (ii) $(\mu_3\delta_2 - \mu_2\delta_1) > 0$

(i)	$h(-\infty)$	$h(B_{41})$	$h(B_{21})$	$h(B_{12})$	$h(B_{13})$	$h(0)$	$h(B_{22})$	$h(B_{61})$	$h(+\infty)$
Signal	+	+	−	+	−	−	+/−	−	+
(ii)	$h(-\infty)$	$h(B_{41})$	$h(B_{21})$	$h(B_{12})$	$h(B_{13})$	$h(B_{61})$	$h(0)$	$h(B_{22})$	$h(+\infty)$
Signal	−	+	−	+	+	+	−	+/−	−

Proposition 2 (One solution of $h(B) = 0$ **in** $(0, B_{22})$) *Suppose that* $\alpha_1\mu_1 < \alpha_{24}$, $\alpha_3\mu_1 < \alpha_{41}$, $\alpha_1\mu_1 < \alpha_2 < \alpha_{24}$ *and* $\alpha_3\mu_1 < \alpha_4 < \alpha_{41}$, *where* α_{24} *is given by Eq. (7) and* α_{41} *is defined in (8). Then,* $h(B) = 0$ *has only one solution in* $(0, B_{22})$ *when* $\sigma < \sigma_1$.

Proof We know that if $\alpha_2 < \alpha_{24}$, then $B_{11} > B_{22}$ (for details and demonstration see Delboni (2015)). Besides that, the function (13) evaluated in B_{22} gives us

$$h(B_{22}) > 0 \Leftrightarrow \alpha_4 < \frac{\mu_3\eta_2\delta_2(B_{11} - B_{22})(1 - \sigma + B_{22})}{\eta_1\delta_1(\sigma_1 - \sigma + \sigma_1 B_{22})},$$

noticing that B_{22} depends only on the parameters $\mu_1, \mu_2, \gamma, \sigma$ and α_2.

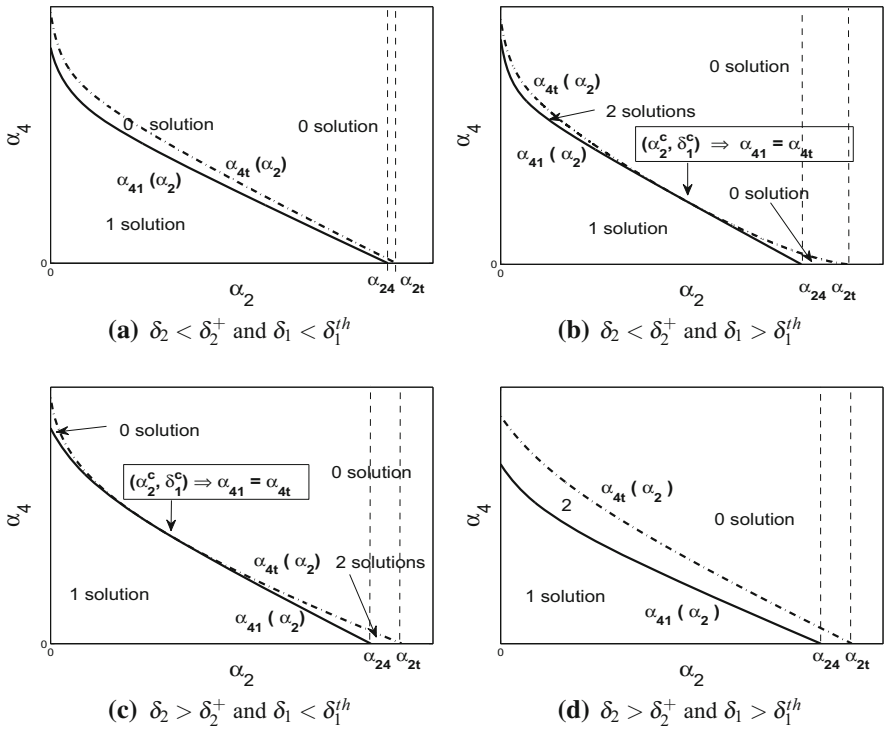
From Table 3, case (i), we verified that if $\alpha_2 < \alpha_{24}$ and $\alpha_4 < \alpha_{41}$, then $h(B_{22}) > 0$ and one signal variation occurs in the interval $(0, B_{22})$, other in (B_{22}, B_{61}), and four additional signal variations. Thus, there is only one viable solution when $\sigma < \sigma_1$ and $(\mu_3\delta_2 - \mu_2\delta_1) < 0$. Similarly, it can be seen in the case (ii) that there is only one viable solution when $(\mu_3\delta_2 - \mu_2\delta_1) > 0$. □

The case $\sigma = 0$ and $\gamma = 0$ represents the existence of optimal conditions of pH, temperature, etc., for a strain of LAB, which is very well suited to the food. A modeling with these hypotheses has been considered in a previous study (Delboni and Yang 2008). Considering in the model (2), we have the equilibrium points $\mathbf{E_0}$ and $\mathbf{E_c}$ (both always unstable), $\mathbf{E_s}$ (conditions for the stability in Theorem 3), and the internal equilibrium which exists if the roots of $h(B)$ is positive and $B < B_{22}$, where $B_{22} = \alpha_2\sigma_1/\mu_2$. The conditions for the existence of only one viable internal equilibrium are given in Proposition 2. If these assumptions are not satisfied, zero or two biological viable internal equilibria are possible, according to the conditions for δ_1 and δ_2 presented before.

When the assumptions of Proposition 2 are not satisfied ($\alpha_2 > \alpha_{24}$ and/or $\alpha_4 > \alpha_{41}$), in order to determine the number of viable solutions of $h(B) = 0$, we must study how many times the curves of functions $f(B)$ and $g(B)$ intersect in the interval $(0, B_{22})$. Therefore, diagrams are drawn in the parameters space $\alpha_2 \times \alpha_4$, which are shown in Fig. 1.

For each α_2 fixed, $(\alpha_4, B) = (\alpha_{4t}(\alpha_2), B_{4t}(\alpha_2))$ is the tangent point of the curves $f(B)$ and $g(B)$, i.e., it is the solution of the non-linear system:

$$\begin{cases} f(B) = g(B) \\ \dfrac{df(B)}{dB} = \dfrac{dg(B)}{dB}. \end{cases} \tag{16}$$



(a) $\delta_2 < \delta_2^+$ and $\delta_1 < \delta_1^{th}$ **(b)** $\delta_2 < \delta_2^+$ and $\delta_1 > \delta_1^{th}$

(c) $\delta_2 > \delta_2^+$ and $\delta_1 < \delta_1^{th}$ **(d)** $\delta_2 > \delta_2^+$ and $\delta_1 > \delta_1^{th}$

Fig. 1 Diagrams showing in the regions of the graph $\alpha_2 \times \alpha_4$ the quantity of solutions of $h(B) = 0$ in the interval $(0, B_{22})$, when $\sigma < \sigma_1$. For $\alpha_2 = \alpha_2^c$ and $\delta_1 = \delta_1^c$, the curves α_{41} and α_{4t} are tangent. The value α_2^c divides the region between the curves α_{41} and α_{4t} with 0 or 2 viable solutions (**b**, **c**)

From the behavior of the functions $f(B)$ and $g(B)$, we notice that it is not possible that both curves tangency when $B > 0$ and $f(B_{22}) > g(B_{22})$ (which must imply $\alpha_4 < \alpha_{41}$), simultaneously. That is, it is not possible that the Eq. (16) has solution such that $\alpha_{4t} < \alpha_{41}$.

If $\alpha_2 < \alpha_{24}$ and $\alpha_4 = \alpha_{41}$, then $f(B_{22}) = g(B_{22})$. We analyze the equation $f'(B_{22}) - g'(B_{22}) = 0$ in those conditions. Therefore, we determine conditions for which the curve $\alpha_{4t}(\alpha_2)$ is tangent to the curve $\alpha_{41}(\alpha_2)$, that is, under which circumstances $\alpha_{41} = \alpha_{4t}$.

Substituting α_4 by α_{41}, and calculating the derivative of $f(B) - g(B)$ at B_{22}, we have

$$f'(B_{22}) - g'(B_{22}) = (\sigma_1 - \sigma + \sigma_1 B_{22})^{-1} \times h_5(B_{22}) B_{22} \times \Gamma_2(\delta_1),$$

with $h_5(B_{22}) = c_{22} B_{22} + c_{12}$ and $\Gamma_2(\delta_1) = \Gamma_n - \delta_1 \Gamma_d$. The equation $f'(B_{22}) - g'(B_{22}) = 0$ is satisfied if $\Gamma_2(\delta_1) = 0$, that is, if $\delta_1 = \delta_1^c = \Gamma_n / \Gamma_d$, where:

$$\begin{cases} \Gamma_n = \mu_3 h_5(B_{22})(\sigma_4 - \eta_2\delta_2 B_{22})(\sigma_1 - \sigma + \sigma_1 B_{22})B_{22} \\ \qquad -\mu_3 h_5(B_{22})(1 - \sigma + B_{22})[\sigma_4\sigma_1 + \eta_2\delta_2(\sigma_1 - \sigma)]B_{22} \\ \qquad -\mu_3(1 - \sigma + B_{22})(\sigma_1 - \sigma + \sigma_1 B_{22})h_1'(B_{22}) \\ \Gamma_d = -(\sigma_4 - \eta_2\delta_2 B_{22})(\sigma_1 - \sigma + \sigma_1 B_{22})(1 - \sigma + B_{22})h_1'(B_{22}). \end{cases}$$

Notice that $\Gamma_d > 0$ due to $h_1'(B_{22}) = 2c_{21}B_{22} + c_{11} < 0$ and $\alpha_2 < \alpha_{24}$, resulting that the signal of δ_1^c depends only on the signal of Γ_n. When $\alpha_2 \to 0$, we have $B_{22} \to 0$, and

$$\lim_{\alpha_2 \to 0} \delta_1^c = \lim_{\alpha_2 \to 0} \frac{\Gamma_n}{\Gamma_d} = \frac{\mu_3}{\sigma_4} = \delta_1^{th}.$$

If $\alpha_2 \to \alpha_{24}$, we have $B_{22} \to B_{11}$, and

$$\lim_{\alpha_2 \to \alpha_{24}} \Gamma_n = \frac{\mu_3(1 - \sigma + B_{11})}{\eta_2^2\delta_2^2} \times \tilde{\Gamma}(\delta_2) \quad \text{and} \quad \lim_{\alpha_2 \to \alpha_{24}} \Gamma_d = 0.$$

By analyzing the function $\tilde{\Gamma}(\delta_2) = m_{32}\delta_2^3 + m_{22}\delta_2^2 + m_{12}\delta_2 + m_{02}$, where

$$\begin{cases} m_{32} = -\eta_2^2\sigma_4(\sigma_1 - \sigma)k_1 \\ m_{22} = \eta_2 k_1[\mu_2\eta_2(\sigma_1 - \sigma) - \sigma_4^2\sigma_1] - \sigma_4^2\eta_2(\sigma_1 - \sigma)(1 + \sigma_1\gamma) \\ m_{12} = \sigma_4(1 + \sigma_1\gamma)[2\mu_2\eta_2(\sigma_1 - \sigma) - \sigma_4^2\sigma_1] \\ m_{02} = \sigma_4^2\sigma_1\mu_2(1 + \sigma_1\gamma), \end{cases}$$

and $k_1 = [(1-\sigma) - \gamma(\sigma - \sigma_1)]$, it is not possible to impose conditions so that $m_{22} > 0$ and $m_{12} < 0$, simultaneously. Therefore, the positive solution δ_2^+ of $\tilde{\Gamma}(\delta_2) = 0$ is unique. We conclude that (a) if $0 < \delta_2 < \delta_2^+$, then $\lim_{\alpha_2 \to \alpha_{24}} \delta_1^c = +\infty$, and (b) if $\delta_2 > \delta_2^+$, then $\lim_{\alpha_2 \to \alpha_{24}} \delta_1^c = -\infty$.

Summarizing, considering the interval for α_2 given by $(0, \alpha_{24})$, we have that:

(i) Suppose that $\delta_2 < \delta_2^+$. If $\delta_1 < \delta_1^{th}$, the curves $\alpha_{41}(\alpha_2)$ and $\alpha_{4t}(\alpha_2)$ are not tangent. If $\delta_1 > \delta_1^{th}$, the curves are tangent at $(\alpha_2^c, \delta_1^c(\alpha_2^c))$ and, moreover, $\delta_1^c \to +\infty$ when $\alpha_2 \to \alpha_{24}$.

(ii) Suppose that $\delta_2 > \delta_2^+$. If $\delta_1 < \delta_1^{th}$ the curves $\alpha_{41}(\alpha_2)$ and $\alpha_{4t}(\alpha_2)$ are tangent at $(\alpha_2^c, \delta_1^c(\alpha_2^c))$ and, moreover, $\delta_1^c \to -\infty$ for when $\alpha_2 \to \alpha_{24}$. Therefore, $\alpha_2^c < \alpha_{2L} < \alpha_{24}$, where α_{2L} is such that $\delta_1^c = 0$, and $\forall\ \delta_1 > 0$ and $\alpha_{2L} < \alpha_2 < \alpha_{24}$, the curves are not tangent. For $\delta_1 > \delta_1^{th}$, the curves are not tangent.

Using the analysis given so far for the case $\sigma < \sigma_1$, it is possible to determine regions, in the parameters space $\alpha_2 \times \alpha_4$, where $h(B) = 0$ has 0, 1 or 2 solutions in the interval $(0, B_{22})$ (Fig. 1). Denoting by B_a, B_b, B_c, B_d, B_e and B_f the solutions of $f(B) = 0$, we have that: (a) if $(\mu_3\delta_2 - \mu_2\delta_1) < 0$, then the negative solutions are $B_a = B_{41}$, $B_b \in (B_{21}, 0)$, $B_c = B_{51}$, and the positives are $B_d = B_{61} > B_{22}$ and $B_e = B_{22}$, and (b) if $(\mu_3\delta_2 - \mu_2\delta_1) > 0$, the negative solutions are $B_a = B_{41}$, $B_b \in (B_{21}, B_{12})$, $B_c = B_{51}$, $B_d = B_{61}$, and the positive is $B_e = B_{22}$. Thus:

(i) Assuming $\delta_2 > \delta_2^+$, then $B_f \in (0, B_{22})$ and consequently $f(B)$ and $g(B)$ intersect twice in $(0, B_{22})$ for $\alpha_4 < \alpha_{4t}$, being tangent at $\alpha_4 = \alpha_{4t}$, and do not intersect for $\alpha_4 > \alpha_{4t}$.
(ii) Assuming $\delta_2 < \delta_2^+$, then $B_f \in (B_{22}, +\infty)$. In this case, as $f(0) < 0$, there is not solution in $(0, B_{22})$, and we have $f(B) < 0$, while $g(B) > 0$ in this interval.

For $\alpha_{24} < \alpha_2 < \alpha_{2t}$, the above considerations follow except that $B_e > B_{22}$ if $\delta_2 < \delta_2^+$, and $B_e < B_{22}$ if $\delta_2 > \delta_2^+$. If $\alpha_2 > \alpha_{2t}$, then $f(B) < 0$, while $g(B) > 0$ in $(0, B_{22})$.

For $\sigma < \sigma_1$, from the dynamic behavior of the system (2), we will analyze and interpret biologically the four different situations represented in the graphs of Fig. 1.

Listeria interacting weakly with lactic acid and bacteriocin ($\delta_1 < \delta_1^{th}$ *and* $\delta_2 < \delta_2^+$)

Notice that the threshold α_{41} as a function of α_2 is inversely proportional to δ_1, and $\alpha_{24}(\delta_2)$ is a decreasing function with respect to δ_2. Therefore, smaller the values of δ_1 and δ_2, greater the area under the curve $\alpha_{41}(\alpha_2)$, where Listeria coexists with LAB. Then, $\alpha_4 > \alpha_{41}$ represents a very high lactic acid and/or bacteriocin production. Weak interaction means lower reduction of lactic acid and bacteriocin by Listeria due to the terms $-\delta_1 AC$ and $-\delta_2 BC$, respectively. Consequently, there will be too high concentration of these metabolites available to eliminate Listeria, which explains the extinction if $\alpha_4 > \alpha_{41}$.

Hereafter, we consider $(\alpha_2^c, \delta_1^c(\alpha_2^c))$ fixed, such that at this point $\alpha_{41} = \alpha_{4t}$.

Listeria interacting weakly with bacteriocin, but strongly with lactic acid ($\delta_2 < \delta_2^+$ *and* $\delta_1 > \delta_1^{th}$)

(a) If $\alpha_2 < \alpha_2^c$, there is a region between the curves $\alpha_{41}(\alpha_2)$ and $\alpha_{4t}(\alpha_2)$ such that there are two internal equilibria. In this case, we have low bacteriocin production rate proportional to the LAB. As the concentration of lactic acid also decreases due to the term $-\delta_1 AC$, it is necessary sufficiently large acid production to completely eliminate Listeria, i.e., $\alpha_4 > \alpha_{4t}$.
(b) If $\alpha_2 > \alpha_2^c$, due to increased production rate of bacteriocin, it is possible to eliminate C even with lower production rate of lactic acid α_4. This is because high production rate of bacteriocin and low interaction rate between Listeria and bacteriocin represent high bacteriocin concentration available to interact and eliminate Listeria.

Listeria interacting strongly with bacteriocin, but weakly with lactic acid ($\delta_2 > \delta_2^+$ *and* $\delta_1 < \delta_1^{th}$)

(a) If $\alpha_2 < \alpha_2^c$, as δ_1 is small, it follows that $\alpha_4 > \alpha_{41}$ represents a very high rate of acid production, which is able to extinguish the population of Listeria.
(b) If $\alpha_2 > \alpha_2^c$, the effect of lactic acid on the bacteria C is negligible, because of low values of α_4 and δ_1. Although $\alpha_2 > \alpha_2^c$ represents higher bacteriocin production rate, the availability in the environment is decreased because of the term $-\delta_2 BC$. Therefore, in the region between the curves $\alpha_{41}(\alpha_2)$ and $\alpha_{4t}(\alpha_2)$ still coexist C and S. The complete elimination is possible by increasing the bacteriocin production rate above $\alpha_2 > \alpha_{2t}$, or lactic acid production rate above $\alpha_4 > \alpha_{4t}$, increasing the availability of antimicrobial metabolites.

Listeria interacting strongly with bacteriocin and lactic acid ($\delta_1 > \delta_1^{th}$ *and* $\delta_2 > \delta_2^+$)

As the interaction rates δ_1 and δ_2 are high, although the concentration of C decreases, also decreases the concentration of lactic acid and bacteriocin available due to terms $-\delta_1 AC$ and $-\delta_2 BC$. Thus, we must increase the lactic acid production rate above $\alpha_4 > \alpha_{4t}$, or increase the bacteriocin production rate above $\alpha_2 > \alpha_{2t}$, in order to completely eliminate Listeria.

From Proposition 2 and discussions done so far about roots of $h(B) = 0$, we summarize all the information in Remark 3.

Remark 3 When $\sigma < \sigma_1$, the sixth-degree polynomial equation $h(B) = 0$ has 0, 1 or 2 solutions in $(0, B_{22})$, according to conditions for the parameters δ_1, δ_2, α_2 and α_4 already established.

3.3.2 $\sigma > \sigma_1$. Viable Solutions Are Such that $B \in (B_{12}, B_{22})$, for $\alpha_2 > \alpha_2^+$.

For $\sigma > \sigma_1$, it is possible to determine conditions for the thresholds related to bacteriocin in order to establish the coexistence equilibrium points. Assuming $\sigma_4 > 0$ and $\sigma_1 > 0$, internal equilibria can be determined taking into account bacteriocin production rate α_2 and the interaction rate with Listeria, given by δ_2.

By comparing above thresholds for α_2 (given by (6) and (7)), one threshold for δ_2 is given by

$$\delta_{2a} = \sigma_4 \times \frac{1}{\eta_2} \times \frac{\sigma_1}{(\sigma - \sigma_1)}. \tag{17}$$

Details and demonstrations can be seen in Delboni (2015).

If, from the equation $dS/dt = 0$, we isolate B and replace it into the equation for C, we get for $\delta_2 > \delta_{2a}$,

$$\frac{dC}{dt} = \frac{[\eta_2(\sigma - \sigma_1)(\delta_{2a} - \delta_2) - \eta_2\delta_2(1 - \sigma)S]C}{(\sigma_1 - S)} - C^2 - \delta_1 AC < 0.$$

For the threshold δ_{2a}, given by Eq. (17), σ_4 is the net reproductive rate of Listeria, $1/\eta_2$ is the average number of bacteriocin molecules necessary for disable a cell C, $\sigma_1/(\sigma - \sigma_1) = 1/B_{13}$ can be understood as the effective action of the bacteriocin, because B_{13} is the threshold condition for the equilibrium point be biologically feasible, then the number $1/\eta_2 B_{13}$ represents how many cells of Listeria are effectively disabled by bacteriocin at equilibrium. Concluding, δ_{2a} measures the proliferation risk of C in the presence of bacteriocin.

Remark 4 Suppose that $\sigma_1 < \sigma < 1$. The relative position of the roots of $h_1(B)$, $f_1(B)$ and $g(B)$ (given by (15)) is presented in Table 4.

In Lemma 1, we present conditions that guarantee the absence of biologically feasible internal equilibrium points.

Lemma 1 (Absence of viable solutions) *Suppose that one of the following conditions is satisfied:*

Table 4 Relative position of the roots of $h_1(B)$, $f_1(B)$ and $g(B)$ (given by (15)), considering $\sigma_1 < \sigma < 1$ in each interval for the parameters δ_2 and α_2

Hypothesis	Interval for δ_2	Interval for α_2	Relative position for B_{ij}
(1)	$0 < \delta_2 < \delta_{2d}$	$\alpha_2^+ < \alpha_2 < \alpha_{24}$	$B_{13} < B_{12} < B_{22} < B_{11}$
(2)		$\alpha_2 > \alpha_{24}$	$B_{13} < B_{12} < B_{11} < B_{22}$
(3)	$\delta_{2d} < \delta_2 < \delta_{2a}$	$\alpha_2^+ < \alpha_2 < \alpha_{24}$	$B_{13} < B_{11} < B_{12} < B_{22}$
(4)		$\alpha_2 > \alpha_{24}$	$B_{13} < B_{12} < B_{11} < B_{22}$
(5)	$\delta_2 > \delta_{2a}$	$\alpha_2 > \alpha_2^+$	$B_{11} < B_{13} < B_{12} < B_{22}$

(i) $\delta_{2d} < \delta_2 < \delta_{2a}$ *and* $\alpha_2^+ < \alpha_2 < \alpha_{24}$*; or*
(ii) $\delta_2 > \delta_{2a}$ *and* $\alpha_2 > \alpha_2^+$,

with thresholds presented in Eqs. (6), (7), (9) and (17). Then, $h(B) = 0$ does not have solutions in (B_{12}, B_{22}).

Proof In Table 4 ,we can see that if one of the assumptions is satisfied, then $B_{11} < B_{12} < B_{22}$ and $f_2(B) > 0$ in the interval (B_{12}, B_{22}). The function $f_1(B)$ has dominant term $-\eta_2\delta_2^2(1+\sigma_1\gamma)B^3$, then: $\lim_{B\to-\infty} f_1(B) = +\infty$, $\lim_{B\to+\infty} f_1(B) = -\infty$, and the solutions of $f_1(B) = 0$ are B_{21}, 0 and B_{11}, all smaller than B_{12}. Therefore, $f_1(B) < 0$ in the interval (B_{12}, B_{22}). Consequently, the curves $f_1(B)$ and $f_2(B)$ do not intersect in this interval and it follows that $f_4(B) = f_1(B) - h_1(B) < 0$.

Consider the equation

$$f_2(B) = (\mu_3\delta_1 - \mu_2\delta_2)(1+\sigma_1\gamma)(B - B_{41})(B - B_{51})(B - B_{61}) = 0,$$

with solutions $B_{41} < 0$, B_{51} and B_{61}. The dominant term is given by $(\mu_3\delta_1 - \mu_2\delta_2)(1+\sigma_1\gamma)B^3$, such that $f_2(0) = -\alpha_2\delta_1(1-\sigma)(\sigma-\sigma_1) < 0$. We know that:

(i) If $(\mu_3\delta_1 - \mu_2\delta_2) > 0$, then $\lim_{B\to-\infty} f_2(B) = -\infty$ and $\lim_{B\to+\infty} f_2(B) = +\infty$. In this case, we conclude that $f_2(B) > 0$ for all $B > B_{61}$, and consequently, as $B_{61} < B_{12}$, it applies for the interval (B_{12}, B_{22}).
(ii) Assuming $(\mu_3\delta_1 - \mu_2\delta_2) < 0$, then $\lim_{B\to-\infty} f_2(B) = +\infty$ and $\lim_{B\to+\infty} f_2(B) = -\infty$. We conclude that $f_2(B) > 0$ for all $B \in (B_{51}, B_{61}) \supset (B_{12}, B_{22})$.

Considering that $f_4(B) < 0$ and $f_2(B) > 0$, we conclude that $f(B) = f_4(B) \times f_2(B) < 0$ for all $B \in (B_{12}, B_{22})$. The function

$$g(B) = \eta_1\delta_1\alpha_4 \times (\sigma_1 - \sigma + \sigma_1 B) \times [h_5(B)]^2 \times B^2$$

is positive when $\sigma > \sigma_1$ and $B > B_{13}$. As $B_{13} < B_{12}$, it follows that $g(B) > 0$, and as $f(B) < 0$, the graphs of these functions do not intercept when $B \in (B_{12}, B_{22})$. □

In Lemma 1, we established the conditions that control the growth of C, which were $\delta_{2d} < \delta_2 < \delta_{2a}$ and $\alpha_2^+ < \alpha_2 < \alpha_{24}$. As interactions between bacteriocin and Listeria occur, both concentrations decrease. So δ_2 is high enough to eliminate C, but

α_2 should also be high enough so that the production of B exceeds elimination due to term $-\delta_2 BC$. The threshold α_{24} is positive and increasing for $\delta_2 \in (\delta_{2d}, \delta_{2a})$ and tends to infinity when $\delta_2 \to \delta_{2a}$. The threshold α_2^+ does not depend on δ_2 and is an increasing function for $\sigma \in (\sigma_1, 1)$.

Studying the signals of $h(B_{12})$ and $h(B_{22})$, we determine conditions for the existence of even number or odd number of internal equilibria in the interval (B_{12}, B_{22}). This analysis is presented in the next proposition.

Proposition 3 (Signal of $h(B_{12})$ and $h(B_{22})$) *Using the thresholds for δ_2 and α_2 presented in* Eqs. (6), (7), (9) *and* (17)*, plus Remark 4 and the thresholds for α_4 defined as*

$$\alpha_{41}=\frac{\mu_3\eta_2\delta_2(B_{11}-B_{22})(B_{22}-B_{41})}{\eta_1\delta_1\sigma_1(B_{22}-B_{13})} \quad \textit{and} \quad \alpha_{42}=\frac{\mu_3\eta_2\delta_2(B_{11}-B_{12})(B_{12}-B_{41})}{\eta_1\delta_1\sigma_1(B_{12}-B_{13})},$$

we conclude that $h(B) = 0$ has at least one biologically feasible solution, if one of the following conditions is satisfied:

(i) $\delta_2 < \delta_{2a}$, $\alpha_2 > \max\{\alpha_1\mu_1, \alpha_{24}\}$, $\alpha_3\mu_1 < \alpha_{42}$ *with* $\alpha_3\mu_1 < \alpha_4 < \alpha_{42}$*; or*
(ii) $\delta_2 < \delta_{2d}$, $\alpha_1\mu_1 < \alpha_{24}$ *with* $\max\left\{\alpha_1\mu_1, \alpha_2^+\right\} < \alpha_2 < \alpha_{24}$, $\alpha_3\mu_1 < \alpha_{42}$, *and* $\max\{\alpha_3\mu_1, \alpha_{41}\} < \alpha_4 < \alpha_{42}$.

However, if one of the following conditions is satisfied, the equation $h(B) = 0$ does not have solution or has an even number of solutions in the interval (B_{12}, B_{22}):

(iii) $\delta_2 < \delta_{2d}$, $\alpha_1\mu_1 < \alpha_{24}$ *with* $\max\left\{\alpha_1\mu_1, \alpha_2^+\right\} < \alpha_2 < \alpha_{24}$, *and: (a)* $\alpha_4 > \max\{\alpha_3\mu_1, \alpha_{42}\}$, *or (b)* $\alpha_3\mu_1 < \alpha_{41}$ *with* $\alpha_3\mu_1 < \alpha_4 < \alpha_{41}$*; or*
(iv) $\delta_2 < \delta_{2a}$, $\alpha_2 > \max\{\alpha_{24}, \alpha_1\mu_1\}$ *with* $\alpha_4 > \max\{\alpha_3\mu_1, \alpha_{42}\}$.

Proof The expression $h(B_{12})$ is

$$h(B_{12}) = \sigma_1\eta_1\delta_1 \times (B_{12} - B_{13}) \times (\alpha_{42} - \alpha_4) \times [h_5(B_{12})]^2 \times [B_{12}]^2,$$

with $[h_5(B_{12})]^2 \times [B_{12}]^2 > 0$. Notice that if $B_{11} < B_{12}$, then $h(B_{12}) < 0$ because $\alpha_{42} < 0$. If $B_{11} > B_{12}$ and $\alpha_4 < \alpha_{42}$, then $h(B_{12}) > 0$, and if $\alpha_4 > \alpha_{42}$, then $h(B_{12}) < 0$.

Now the signal for $h(B_{22})$,

$$h(B_{22}) = \sigma_1\eta_1\delta_1 \times (B_{22} - B_{13}) \times (\alpha_{41} - \alpha_4) \times [h_5(B_{22})]^2 \times [B_{22}]^2,$$

is studied. If $B_{11} < B_{22}$, then $h(B_{22}) < 0$ for all positive α_4. If $B_{11} > B_{22}$ and $\alpha_4 < \alpha_{41}$, then $h(B_{22}) > 0$, and $h(B_{22}) < 0$ if $\alpha_4 > \alpha_{41}$.

Under above assumptions and using Remark 4, we conclude that $h(B_{12})$ and $h(B_{22})$ have opposite signs, ensuring at least one biologically feasible solution for $h(B) = 0$.

With the hypotheses (iii) and (iv), it follows that $h(B_{12})$ and $h(B_{22})$ have the same sign. We conclude that if there is biologically feasible solution for $h(B) = 0$, then there will be an even number of solutions. □

Table 5 Signal of the function $h(B)$, when $\sigma > \sigma_1$ and $(\mu_3\delta_2 - \mu_2\delta_1) > 0$, for roots of $h_1(B)$, $f_1(B)$, $f_2(B)$ and $g(B)$ (given by (15)), and number of solutions of $h(B) = 0$ in (B_{12}, B_{22}), for different conditions of the parameters δ_2 (given by (9) and (17)) and α_2 (given by (6) and (7)) and hypotheses for α_4 (see Proposition 3)

Hypothesis	$h(-\infty)$	$h(B_{41})$	$h(0)$	$h(B_{61})$	$h(B_{12})$	$h(B_{22})$	$h(+\infty)$	Solutions
Condition (i): $\delta_2 < \delta_{2a}$ and $\alpha_2 > \max\{\alpha_1\mu_1, \alpha_{24}\}$								
(a) $\alpha_3\mu_1 < \alpha_4 < \alpha_{42}$	−	+	−	+	+	−	−	1 or 3
(b) $\alpha_4 > \alpha_{42}$	−	+	−	+	−	−	−	0 or 2
Condition (ii): $\delta_2 < \delta_{2d}$ and $\alpha_1\mu_1 < \alpha_{24}$ with $\max\left\{\alpha_1\mu_1, \alpha_2^+\right\} < \alpha_2 < \alpha_{24}$								
(a) $\alpha_3\mu_1 < \alpha_4 < \alpha_{41}$	−	+	−	+	+	+	−	0 or 2
(b) $\alpha_{41} < \alpha_4 < \alpha_{42}$	−	+	−	+	+	−	−	1 or 3
(c) $\alpha_4 > \alpha_{42}$	−	+	−	+	−	−	−	0 or 2

Table 6 Signal of the function $h(B)$, when $\sigma > \sigma_1$ and $(\mu_3\delta_2 - \mu_2\delta_1) < 0$, for roots of $h_1(B)$, $f_1(B)$, $f_2(B)$ and $g(B)$ (given by (15)), and number of solutions of $h(B) = 0$ in (B_{12}, B_{22}), for different conditions of the parameters δ_2 (given by (9) and (17)) and α_2 (given by (6) and (7)) and hypotheses for α_4 (see Proposition 3)

Hypothesis	$h(-\infty)$	$h(B_{41})$	$h(0)$	$h(B_{12})$	$h(B_{22})$	$h(B_{61})$	$h(+\infty)$	Solutions
Condition (i): $\delta_2 < \delta_{2a}$ and $\alpha_2 > \max\{\alpha_1\mu_1, \alpha_{24}\}$								
(a) $\alpha_3\mu_1 < \alpha_4 < \alpha_{42}$	+	+	−	+	−	−	+	1 or 3
(b) $\alpha_4 > \alpha_{42}$	+	+	−	−	−	−	+	0, 2 or 4
Condition (ii): $\delta_2 < \delta_{2d}$ and $\alpha_1\mu_1 < \alpha_{24}$ with $\max\left\{\alpha_1\mu_1, \alpha_2^+\right\} < \alpha_2 < \alpha_{24}$								
(a) $\alpha_3\mu_1 < \alpha_4 < \alpha_{41}$	+	+	−	+	+	−	+	0 or 2
(b) $\alpha_{41} < \alpha_4 < \alpha_{42}$	+	+	−	+	−	−	+	1 or 3
(c) $\alpha_4 > \alpha_{42}$	+	+	−	−	−	−	+	0, 2 or 4

Using the results of Proposition 3, we present in the following corollaries the conditions that determine the number of solutions in the interval (B_{12}, B_{22}) for $(\mu_3\delta_2 - \mu_2\delta_1) > 0$ and $(\mu_3\delta_2 - \mu_2\delta_1) < 0$, respectively.

Corollary 2 (Solutions in (B_{12}, B_{22}) when $(\mu_3\delta_2 - \mu_2\delta_1) > 0$) *Suppose that* $(\mu_3\delta_2 - \mu_2\delta_1) > 0$. *Using thresholds for* δ_2 *and* α_2 *from Remark 4, plus the thresholds of* α_4 *and the sign of* $h(B_{12})$ *and* $h(B_{22})$ *determined in Proposition 3, the number of biologically feasible solutions (in the interval* (B_{12}, B_{22})*) is presented in Table 5.*

Corollary 3 (Solutions in (B_{12}, B_{22}) when $(\mu_3\delta_2 - \mu_2\delta_1) < 0$) *Suppose that* $(\mu_3\delta_2 - \mu_2\delta_1) < 0$. *Using thresholds for* δ_2 *and* α_2 *from Remark 4, plus the thresholds of* α_4 *and the sign of* $h(B_{12})$ *and* $h(B_{22})$ *determined in Proposition 3, the number of solutions in* (B_{12}, B_{22}) *is presented in Table 6.*

From Corollaries 2 and 3, and with the analysis done so far about roots of $h(B) = 0$, we summarize all the information in Remark 5.

Remark 5 When $\sigma_1 < \sigma < 1$, the sixth-degree equation $h(B) = 0$ can have a maximum of three solutions in (B_{12}, B_{22}) when $(\mu_3\delta_2 - \mu_2\delta_1) > 0$, and a maximum of four viable solutions if $(\mu_3\delta_2 - \mu_2\delta_1) < 0$, according to conditions for the parameters δ_1, δ_2, α_2 and α_4 already established.

4 Conclusions

Aiming to study quantitatively the biological control as a technique of food conservation, we developed a mathematical model to describe the interaction between bacteriocin-producing lactic acid bacteria and Listeria in the food. The differential of the proposed model is the inclusion of quorum sensing in the growth rate of lactic acid bacteria and also in the bacteriocin production rate. The goal was to find the thresholds which determine the existence of multiple equilibria and analyze their stability.

Explanation of the coexistence of competing species is a major challenge in ecology of communities (Riley 2011). The use of theoretical models is important to obtain information about the study at the community level, helping to identify interface regions between growth and no growth, for example. Thus, theoretical modeling can provide general explanations for specific observed results.

Suppose $\sigma < \sigma_1$. This means that the cost to bacteriocin production is low, probably due to fact that LAB are in an adequate environment, with favorable conditions for growth and bacteriocin production, as pH and temperature. In this case, we emphasize the importance of Proposition 2, where we found conditions for the existence of only one internal equilibrium point $\mathbf{E_*}$ with both bacteria coexisting. In Theorems 1, 2 and 3, hypotheses were presented, and when those conditions were satisfied, we proved the local stability of the trivial equilibrium $\mathbf{E_0}$ and of the boundary equilibria $\mathbf{E_c}$ and $\mathbf{E_s}$. It is of practical importance the conditions for the stability of the point $\mathbf{E_s}$, where there is no Listeria. However, it is also fundamental the analysis of the existence of internal equilibria, because even when the conditions of Theorem 3 are satisfied (and Theorem 4 when $\sigma > \sigma_1$), we guaranteed only the local stability of equilibrium point $\mathbf{E_s}$. Due to the possibility of multiple internal equilibria, the extinction of Listeria also depends on the initial contamination in these cases [see companion paper (Delboni and Yang, submitted)].

For $\sigma > \sigma_1$, we concluded that the conditions given by Corollary 1 and by Lemma 1 really bear biological sense and explain the non-existence of $\mathbf{E_*}$. It was not possible to study in detail the functions $f(B)$ and $g(B)$ as we did for the case $\sigma < \sigma_1$, because when σ exceeds the threshold σ_1, these functions may have many different behaviors. Then, we did not obtain restrictive conditions for the parameters for each number of viable solutions of $h(B) = 0$ and, consequently, for each number of biologically feasible coexistence equilibria. The possibilities are presented in Tables 5 and 6, and a further paper (see companion paper (Delboni and Yang, submitted)) will present a numerical analysis exploring all these possibilities.

In a companion paper (Delboni and Yang, submitted), we deal with bifurcation diagrams and attracting basins. By doing numerical simulations, we discuss the local stability of multiple equilibria, with particular interest in the internal equilibria. Thus,

we present the importance and usefulness of application of bacteriocin-producing lactic acid bacteria as biological control in food preservation.

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