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An allelopathy based model for the *Listeria* overgrowth phenomenon

Hedia Fgaier [a,*], Martin Kalmokoff [b], Timothy Ells [b], Hermann J. Eberl [a]

[a] Dept. Mathematics and Statistics, University of Guelph, Guelph, ON N1G 2W1, Canada
[b] Atlantic Food and Horticulture Research Center, Agriculture and Agri-Food Canada, Kentville, NS B4N 1J5, Canada

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ABSTRACT

In a standard procedure of food safety testing, the presence of the pathogenic bacterium *Listeria monocytogenes* can be masked by non-pathogenic *Listeria*. This phenomenon of *Listeria* overgrowth is not well understood. We present a mathematical model for the growth of a mixed population of *L. innocua* and *L. monocytogenes* that includes competition for a common resource and allelopathic control of *L. monocytogenes* by *L. innocua* when this resource becomes limited, which has been suggested as one potential explanation for the overgrowth phenomenon. The model is tested quantitatively and qualitatively against experimental data in batch experiments. Our results indicate that the phenomenon of masked pathogens can depend on initial numbers of each population present, and on the intensity of the allelopathic effect. Prompted by the results for the batch setup, we also analyze the model in a hypothetical chemostat setup. Our results suggest that it might be possible to operate a continuous growth environment such that the pathogens outcompete the non-pathogenic species, even in cases where they would be overgrown in a batch environment.

1. Introduction

Listeria monocytogenes is a human pathogen responsible for listeriosis, a foodborne illness that can lead to meningitis, septicemia, spontaneous abortion, perinatal infections and gastroenteritis [5]. While instances of listeriosis are rare and the risk of infection is low for healthy individuals, in those at high risk (elderly, immune-compromised or pregnant) the mortality rate can be as high as 30%. For example, a 2008/09 outbreak that originated in an Ontario meat processing plant caused 22 deaths [15]. *L. monocytogenes* has been frequently isolated from dairy, meat and also vegetable products, in particular processed and packaged foods. The US government has established a "zero tolerance" policy for this pathogen, while in Canada and some European countries the acceptance level is 100 colony forming units per g ready-to-eat (RTE) product with a refrigerated shelf-life of less than 10 days or in RTE foods not supporting the growth of *L. monocytogenes* [9,16]. In addition to the health implications of foods contaminated with this pathogen, it has a tremendous economic consequence for manufacturers, due to costs associated with product recall and decreased sales of implicated products and brands.

Typically, contaminated food contains low numbers of *L. monocytogenes* which cannot be easily detected without prior enrichment to increase cell numbers. An international standard method for *L. monocytogenes* detection in food (ISO11290-1) consists of a two step process where food samples are placed in an enrichment broth which allows *Listeria* species to grow but is detrimental to other microorganisms [17]. This method is reliable if the pathogenic *L. monocytogenes* is the only representative of the *Listeria* genus in the sample. However, this is often not the case and in some instances overgrowth of *L. monocytogenes* by other *Listeria* species can occur if they are also present in the original food sample. In this case, the smaller numbers of the pathogen can remain undetected among the dominating non-pathogens, leading to false negatives [14]. This overgrowth phenomenon in enrichment cultivation has been observed in several studies [4,7,8,20]. Several underlying mechanisms have been proposed to explain it, including competition for limiting nutrients, the physiological state of the cells, and inhibitors that are produced by the bacteria [4]. This overgrowth phenomenon is not well understood, partially because the mathematical models that are routinely used in food microbiology to determine growth kinetics of bacteria are too simple to describe growth limiting processes with sufficient detail.

In this paper we propose a mathematical model for the growth of a mixed population of non-pathogenic *L. innocua* and pathogenic *L. monocytogenes* that includes two of the these mechanisms for *Listeria* overgrowth. The model considers competition of both species for a common resource; when this resource becomes limited, *L. innocua* starts producing a compound that is detrimental for *L. monocytogenes* (allelopathic interaction). The model is tested by quantitative comparison against data sets of batch experiments in the published literature. Our primary finding, obtained by quantitative and qualitative analysis of the model in batch and

* Corresponding author. Tel.: +1 5197259513.
E-mail address: hfgaier@uoguelph.ca (H. Fgaier).

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continuous growth environments, is that with these strains the fate of *L. monocytogenes* in a mixed growth environment is more controlled by the toxins produced by *L. innocua* than by active competition for a shared resource. In particular our results suggest that in the continuous growth system we can have bistability of both semi-trivial equilibria (only one of both species survives) due to the allelopathic growth advantage of *L. innocua*, which can be strong enough to override the principle of competitive exclusion in purely competitive systems [18], where always the species with the lower break-even concentration survives.

2. Mathematical model in a batch setup

2.1. Governing equations

We propose a mathematical model for the growth of a mixed population of *L. innocua* and *L. monocytogenes*. Both species compete for a substrate; limitation of this substrate triggers *L. innocua* to release a compound that is detrimental to *L. monocytogenes*. The model is cast as a system of differential equations for the four dependent variables: density of *L. innocua* (X_1), density of *L. monocytogenes* (X_2), concentration of the growth limiting substrate S, and concentration of the inhibitor C. The governing equations read:

$$\frac{dX_1}{dt} = \alpha_1 X_1 f_1(S), \tag{1}$$

$$\frac{dX_2}{dt} = \alpha_2 f_2(S) X_2 - f_3(C) X_2, \tag{2}$$

$$\frac{dS}{dt} = -\frac{1}{Y_1}\alpha_1 X_1 f_1(S) - \frac{1}{Y_2}\alpha_2 X_2 f_2(S), \tag{3}$$

$$\frac{dC}{dt} = \alpha_1 f_4(S) X_1 - \mu_5 C. \tag{4}$$

In (1) the function $f_1(S)$ denotes the growth rate of X_1 in dependence of the substrate concentration S, similarly $f_2(S)$ in (2) denotes the growth rate of X_2 in dependence of substrate concentration S. Assuming standard Monod kinetics, these are

$$\begin{cases} f_1(S) = \frac{\mu_1 S}{\kappa_1 + S}, \\ f_2(S) = \frac{\mu_2 S}{\kappa_2 + S}. \end{cases} \tag{5}$$

Here μ_1, and μ_2 are maximum growth rates for the substrate per unit mass of bacteria, and κ_1, and κ_2 are half saturation coefficients for the substrates, respectively for X_1 and X_2. If substrate is available in abundance, $S \gg \kappa_{1,2}$, the growth rates are independent of substrate availability and growth rates are approximately constant, i.e. they follow 0th order kinetics. If substrate is limited, $S \ll \kappa_{1,2}$, the growth rate is proportional to the substrate concentration, i.e. follows 1st order kinetics. Growth of both species is due to substrate uptake, modeled by (3). Here $Y_{1,2}$ are yield coefficients that describe the conversion of substrate into biomass.

Bacterial growth curves of batch experiments typically show an initial lag-phase with no or drastically slowed down growth. Commonly this is attributed to physiological recovery of the cells, e.g. after being refrigerated. While this phenomenon usually does not affect the qualitative longterm behavior of the populations, it is an important aspect if the model is to be fitted against experimental data, as will be shown later. Several simple lag-phase models have been proposed in the predictive food microbiology literature, based on different biological considerations, including the Barnayi–Roberts model [1], the Hills–Wright model [10], and the McKellar model [13]. However, it was shown in [6], that all these models can be interpreted and re-formulated as simple logistic equations. Therefore, we have

$$\frac{d\alpha_1}{dt} = \alpha_1 \nu_1 (1 - \alpha_1), \tag{6}$$

$$\frac{d\alpha_2}{dt} = \alpha_2 \nu_2 (1 - \alpha_2), \tag{7}$$

which can be solved to obtain

$$\alpha_1(t) = \frac{\Phi_{0,1} e^{\nu_1 t}}{1 + \Phi_{0,1} e^{\nu_1 t}}, \tag{8}$$

$$\alpha_2(t) = \frac{\Phi_{0,2} e^{\nu_2 t}}{1 + \Phi_{0,2} e^{\nu_2 t}}. \tag{9}$$

Substituting these expressions into (1)–(4), we obtain a four-dimensional non-autonomous model instead of the original six-dimensional autonomous system. The positive parameters $\Phi_{0,1}$ and $\Phi_{0,2}$ are measures of the initial physiological state of the populations of *L. innocua* and *L. monocytogenes*, respectively with a value of ∞ to indicate a perfectly healthy population (capable of immediate maximal growth) and a value of 0 to indicate an irreparable population. The parameters ν_1 and ν_2 are measures of recovery potential for *L. innocua* and *L. monocytogenes*, respectively.

The term $-f_3(C)X_2$ in (2) describes the growth limitation of species X_2 due to toxin C. We assume first order kinetics for this process,

$$f_3(C) = \mu_3 C. \tag{10}$$

The toxin is produced by X_1 when substrate S becomes limited. This is described in (4), where $f_4(S)$ is assumed to follow standard inhibition kinetics,

$$f_4(S) = \frac{\mu_4}{\kappa_1 + S}. \tag{11}$$

Eq. (4) also contains a term describing abiotic decay of the toxin at rate μ_2.

Throughout we assume that all model parameters are positive.

With this in mind, the model given by Eqs. (1)–(4) with coefficient functions (8), (9), (5), (10), (11) is well posed. The model predicts that the population of X_1 producing the toxin grows initially and then reaches a steady state value, while the population X_2 eventually vanishes; it grows initially when there is enough nutrient in the system, but it slows down as the nutrient becomes depleted and as the concentration of toxin increases. This is formalized in the following statement.

Proposition 1 (*Model behavior*). *The system (1)–(4) with coefficient functions (8), (9), (5), (10), (11) and initial data $X_1(0) > 0$, $X_2(0) > 0$, $S(0) > 0$, $C(0) \geqslant 0$ possesses a nonnegative unique solution which is bounded by constants from above. Moreover $X_1(t)$ is a monotonically increasing function and $X_1(t) \to X_{1\infty} > 0$ as $t \to \infty$, $X_2(t)$ is a monotonically decreasing function for large enough t and $X_2(t) \to 0$ as $t \to \infty$, $S(t)$ is monotonically decreasing and $S(t) \to 0$ as $t \to \infty$, and $C(t)$ is monotonic for large enough t and $C(t) \to C_\infty > 0$ as $t \to \infty$.*

Proof. Non-negativity of the solutions to this initial value problem follows with standard arguments, e.g. the invariance theorems in [19]. In the positive cone, the right hand side of (1)–(4) is differentiable, i.e. the system satisfies a Lipschitz condition, which implies existence and uniqueness. Monotonicity of X_1 and S follows directly from (1) and (3) and the non-negativity of the solutions.

From (1)–(3) follows

$$\frac{dX_1}{dt} + \frac{dX_2}{dt} + Y_1 \frac{dS}{dt} = \alpha_2 f_2(S) X_2 \left(1 - \frac{Y_1}{Y_2}\right) - f_3(C) X_2 \tag{12}$$

and