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Bayesian modeling of two- and three-species bacterial competition in milk

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A B S T R A C T

Listeria monocytogenes is a well-known food-borne pathogen and is among the bacteria best adapted to grow at low temperatures. Psychrotrophic spoilage microorganisms present in milk and milk products are primarily in the genus *Pseudomonas*, and their numbers increase during cold storage leading to deterioration and/or spoilage. The nature of the competition in two- or three-species bacterial systems with *L. monocytogenes*, *L. innocua*, and *P. fluorescens* in skimmed milk at 7 or 14°C was studied. The Baranyi growth model was used to estimate the growth rate and the maximum population density of the three microorganisms for each strain in single cultures or in two- or three-strains co-cultures. The highest *Listeria* populations were achieved by pure cultures, decreasing in co-culture with *P. fluorescens* at both temperatures. A modified deterministic logistic model was applied which includes inhibition functions for single cultures, and two- or three-species cultures. A subsequent Bayesian approach was applied for modelling the bacterial interactions. There was not a direct correlation between the growth rate of *P. fluorescens* and its inhibitory effect on *Listeria* species. The use of some species from the natural food microflora to inhibit pathogen growth may be an important tool to enhance the safety of refrigerated foods such as milk and dairy products.

1. Introduction

Listeria monocytogenes is a well-known food-borne pathogen and is among the pathogens best adapted to grow at low temperatures (Farber & Peterkin, 1991). It may occur in raw milk and in the processing environment because of its widespread distribution and association with domestic livestock (Winkowski, Crandall, & Montville, 1993). Its presence in the processing environment increases the potential for post-processing contamination (Carpentier & Cerf, 2011; Farber & Peterkin, 1991; Gandhi & Chikindas, 2007). *L. monocytogenes* can survive and grow at 3 °C in tryptose phosphate broth and at 4 °C in milk (Farber & Peterkin, 1991; Gray & Killinger, 1966); so cold storage of dairy products cannot be relied upon to prevent its growth, should they become contaminated (Farrag & Marth, 1989a, 1989b).

Psychrotrophic spoilage microorganisms present in milk and milk products can come from soil, water, and vegetation and are primarily in the genus *Pseudomonas* (Cousin, 1982), and growth of these organisms during cold storage can lead to deterioration and/or spoilage. *Pseudomonas fluorescens* is an example of a psychrotrophic, Gram-negative spoilage microorganism that *L. monocytogenes* would likely encounter in a variety of refrigerated foods also as a post-processing contaminant (Buchanan & Bagi, 1999).

The use of competitive microbiota to enhance the safety of products has been widely proposed (Gálvez, Abriouel, López, & Omar, 2007; Holzapfel, Geisen, & Schillinger, 1995; Verraes et al., 2013). *P. fluorescens* produces extracellular matrix materials that give secreting cells a positional advantage over competitors (Nadell & Bassler, 2011), which are physically displaced (Schluter, Nadell, Bassler, & Foster, 2015) or cut off from nutrient access (Kim, Racimo, Schluter, Levy, & Foster, 2014). Co-culture experiments without barriers showed which genotypes prevail in mixed cultures (Cornforth & Foster, 2013; Kerr, Riley, Feldman, & Bohannan, 2002; Nadell & Bassler, 2011). Cell-cell contact between bacteria is required for bacterial interactions (Aoki et al., 2005; Avendaño-Pérez & Pin, 2013; Dubey & Ben-Yehuda, 2011; Lemonnier et al., 2008; MacIntyre, Miyata, Kitaoka, & Pukatzki, 2010), demonstrating the need for experiments using co-cultured organisms in systems without physical barriers. In nature, cells commonly mix with cells of different genotypes without physical barriers that impede interactions; and biofilms are one of the most important examples of this phenomenon (Nadell, Drescher, & Foster, 2016).

The interaction of bacteria in food systems has been usually studied in relation to the production of antimicrobial agents such as bacteriocins or acids. A spoilage microorganism can either stimulate, inhibit or have no effect on the growth of the pathogenic species (Buchanan & Bagi, 1999), due to the effect of these agents and/or from other factors. Previous studies have shown that *P. fluorescens* could stimulate (Farrag & Marth, 1989b; Marshall, Andrews, Wells, & Farr, 1992; Marshall & Schmidt, 1988, 1991; Mellefont, McMeekin, & Ross, 2008), inhibit (Al-Zeyara, Jarvis, & Mackey, 2011; Buchanan & Bagi, 1997, 1999; Cheng, Doyle, & Luchansky, 1995; Farrag & Marth, 1989c; Fgaier & Eberl, 2010; Freedman, Kondo, & Willrett, 1989; Mellefont et al., 2008), or have no effect (Farrag & Marth, 1989a; Marshall et al., 1992) on the growth of *L. monocytogenes* depending on variables such as culture media, food matrix, temperature, initial populations, or strains.

The aim of this work was to examine the effect of co-culturing species on the growth/inactivation behavior of two- or three-species bacterial systems with *Listeria monocytogenes*, *L. innocua*, and *Pseudomonas fluorescens* in skimmed milk at 7 or 14 °C; mathematical models of those interactions were developed using deterministic and Bayesian inference techniques.

2. Materials and methods

2.1. Bacterial cultures and inoculation

Listeria monocytogenes and *L. innocua* strains (isolated from a local Spanish milk processing plant; identified by the Faculty of Veterinary Medicine, Universidad de Santiago de Compostela, Spain), and *Pseudomonas fluorescens* CECT 378 (ATCC 13525) were cultured in BHI broth (Difco, BD Diagnostics, Spark, MD, USA) and incubated at 37 °C for 24 h. Bacterial cultures were grown at 37 °C until a population of 10^9 CFU/ml was reached. Serial dilutions of each microorganism were prepared in 0.1% peptone water (Difco). Aliquots (1 ml) from adequate dilutions were added to 250 ml screw-capped Erlenmeyer flasks containing 100 ml of 10% reconstituted sterile skimmed milk (SM), achieving initial populations of ca. 10^3 CFU/ml. The populations were evaluated by spreading onto TSA (Difco) plates and incubating at 37 °C for 48 h.

2.2. Co-cultures and enumeration

A 3 × 2 full factorial experiment with a first 3-level factor (*L. monocytogenes*, *L. innocua*, and *P. fluorescens* strains) and a second 2-level factor (7 or 14 °C) was used. Co-cultures of *L. monocytogenes*, *L. innocua*, and *P. fluorescens* with targeted starting concentration of ca. 10^3 CFU/ml were prepared as described above and stored at 7 or 14 °C. Actual co-culture starting populations of 3.1–3.6, 3.4–4.0, and 3.7–4.3 log CFU/ml were obtained for *L. monocytogenes*, *L. innocua*, and *P. fluorescens*, respectively. The same initial target populations of *L. monocytogenes*, *L. innocua*, and *P. fluorescens* were cultivated in single cultures, and cultures of un-inoculated SM (controls) were also made and stored at the same temperatures.

Cultures were periodically sampled at times up to 50 d exceeding the shelf life period of pasteurized milk and going well into the "best before date" of UHT milk. Sampling times occurred at 0, 3, 6, 9, 12, or 24 h, and 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 20, 24, 28, 32, 40, 48 days at both temperatures. The microorganisms were enumerated as follows: aliquots (0.1 ml) of adequate dilutions were surface-plated onto Listeria Selective Agar Oxford Formulation (Oxoid, Thermo Fisher Scientific Inc., UK), and onto Pseudomonas Agar F or Flo Agar (Difco). Plates were incubated at 35 °C for 48–72 h or 35 °C for 24–48 h for *Listeria* spp. or *P. fluorescens* counting, respectively. When 0.1 ml from 10^{-1} dilution was surfaced-plated, the limit of detection of this technique was 10 CFU/ml (or 1 CFU/0.1 ml). Aliquots of 1 ml were used when needed, achieving a limit of detection of 1 CFU/ml.

L. monocytogenes and *L. innocua* were differentiated by haemolytic activity using the overlay technique (Dominguez et al., 1990; Fernandez-Garayzabal et al., 1992). Briefly, plates previously incubated with the microorganisms were cooled at 4 °C for 2 h and subsequently covered by an 8-ml overlay composed by 37 g/l of BHI broth (Difco), 3 g/l of Bacto agar (Difco), 8 g/l of NaCl, and 50 ml/l of a suspension of Sterile Sheep Blood (Oxoid). The overlay, without the suspension added, was sterilized and stored at 4 °C; just before use, it was boiled, cooled to 45 °C and mixed with the blood suspension. After the addition of the overlay the plates were incubated at 30 °C for 14 h and haemolytic activity was recorded.

2.3. Curve fitting

Plate counts of *L. monocytogenes*, *L. innocua*, and *P. fluorescens* were transformed to decimal logarithmic values. The curves were divided in growth and decline periods. The growth parameters (lag time, maximal growth rate, and maximum population density) were estimated using the DMFit Web Edition, Institute of Food Research, Norwich, UK (Baranyi & Roberts, 1994).

2.4. Logistic modeling of microbial interactions

A deterministic generic primary model (Baranyi & Roberts, 1995) for bacterial cultures can be written as:

$$\frac{dN_t/dt}{N_t} = \frac{d(\ln(N_t))}{dt} = \mu_{\max} \tag{1}$$

where $(dN_t/dt)/N_t$ is the relative or instantaneous growth rate of the microorganism, N_t is the cell concentration in a bacterial culture at time t, and μ_{max} is the maximum growth rate. The Jameson-effect hypothesis (Jameson, 1962; Ross, Dalgaard, & Tienungoon, 2000) states for bacterial co-cultures that (i) many microbial interactions in foods only limit the maximum population density, without any significant effect on the lag time or growth rate, and (ii) the minority population decelerates when the majority or the total population count reaches its maximum. As further detailed in the literature (Cornu, Billoir, Bergis, Beaufort, & Zuliani, 2011), a modified generic primary growth model, also called a Jameson-effect model, can be written as:

$$\frac{dN_t/dt}{N_t} = \frac{d(\ln(N_t))}{dt} = \mu_{\max}\alpha_t f_t \tag{2}$$

where α_t is an adjustment function and f_t is a logistic inhibition function, defined as:

$$\alpha_t = \begin{cases} 0 \; if \; t < \lambda \\ 1 \; if \; t \geq \lambda \end{cases}$$
$$f_t = (1 - (N_t/N_{\max})) \tag{3}$$

where λ is the lag time, and N_{max} is the maximal population density. The logistic deceleration model f_t was modified by Cornu, 2001 for two-species mixed cultures relying on the hypothesis that both populations are similarly inhibited by the same limiting resource, the same waste products and/or by the change in pH (Eq. (4)). We propose a further modification of the logistic deceleration for a three-species mixed culture (Eq. (5)):

$$f_t = 1 - \frac{Na_t + Nb_t}{N_{\max 2spp}} \tag{4}$$

$$f_t = 1 - \frac{Na_t + Nb_t + Nc_t}{N_{\max 3spp}} \tag{5}$$

where Na_t, Nb_t, and Nc_t are the cell concentration of the microorganisms *a*, *b*, or *c* in co-culture at time *t*, and $N_{max\ 2spp}$ or $N_{max\ 3spp}$ are the maximum total population density (including all present species) and consequently the overall carrying capacity of the system from the two- or three-species co-cultured. Therefore, the model can be re-defined for

L. monocytogenes cultures as follows:

$$\frac{dLm/dt}{Lm_t} = \frac{d(\ln(Lm_t))}{dt} = \mu_{Lm}\alpha_t\left(1 - \frac{Lm_t}{Lm_{\max}}\right) \quad (6a)$$

$$\frac{dLm/dt}{Lm_t} = \frac{d(\ln(Lm_t))}{dt} = \mu_{Lm(Li)}\alpha_t\left(1 - \frac{Lm_t + Li_t}{N_{\max}}\right) \quad (6b)$$

$$\frac{dLm/dt}{Lm_t} = \frac{d(\ln(Lm_t))}{dt} = \mu_{Lm(Pf)}\alpha_t\left(1 - \frac{Lm_t + Pf_t}{N_{\max}}\right) \quad (6c)$$

$$\frac{dLm/dt}{Lm_t} = \frac{d(\ln(Lm_t))}{dt} = \mu_{Lm(Li,Pf)}\alpha_t\left(1 - \frac{Lm_t + Li_t + Pf_t}{N_{\max}}\right) \quad (6d)$$

where μ_{Lm} (Eq. (6a)), $\mu_{Lm(Li)}$ (Eq. (6b)), $\mu_{Lm(Pf)}$ (Eq. (6c)), and $\mu_{Lm(Li,Pf)}$ (Eq. (6d)) are the maximal exponential growth rates of *L. monocytogenes* in single cultures or in co-culture with *L. innocua*, *P. fluorescens*, or both, respectively. N_{max} is the maximum total population density achieved by the microorganisms from the two- or three-species co-cultures. Similar approaches to the Eqs. (6a)–(6d) were done for the *L. innocua* (μ_{Li}, $\mu_{Li(Lm)}$, $\mu_{Li(Pf)}$, and $\mu_{Li(Lm,Pf)}$) and *P. fluorescens* (μ_{Pf}, $\mu_{Pf(Lm)}$, $\mu_{Pf(Li)}$, and $\mu_{Pf(Lm,Li)}$) cultures.

Once cultures reached their respective N_{max} values, a decline phase was observed. In order to obtain the best-fitted results, both growth and decline phases were modeled separately. The decline of microorganisms' populations was modeled separately using a modification of Eqs. (6a)–(6d), where the parameter μ was replaced by the negative-sign parameter k.

2.5. Bayesian inference of microbial interactions

As bacterial growth may be influenced by factors outside the experimental design, it is convenient to introduce an error term. Thus, we consider that the observed concentration of bacteria at time t, is N_t^*, where

$$N_t^* = N_t + \varepsilon_t \quad (7)$$

where N_t is the population of *L. monocytogenes*, *L. innocua*, or *P. fluorescens*, in single cultures or in co-culture, and ε_t represents an error term normally distributed with zero mean and constant variance equal to σ_t: $N_t^* \sim \text{Normal}(N_t, \sigma_t)$.

The parameters that must be estimated are the exponential growth rates of the microorganisms in single cultures (μ_{Lm}, μ_{Li}, and μ_{Pf}), the 2-species mixtures ($\mu_{Lm(Li)}$, $\mu_{Lm(Pf)}$, $\mu_{Li(Lm)}$, $\mu_{Li(Pf)}$, $\mu_{Pf(Lm)}$, and $\mu_{Pf(Li)}$,), the 3-species mixtures ($\mu_{Lm(Li,Pf)}$, $\mu_{Li(Lm,Pf)}$, and $\mu_{Pf(Lm,Li)}$), and the standard deviation of errors (σ_t). These parameters are shown in Fig. 1 as circles, while the other terms (constants) are depicted as squares. Decline rates k were also estimated based on the parameters obtained after the use of Eqs. (6a)–(6d) where the parameter μ was replaced by the negative-sign parameter k (see Section 2.4).

The Bayesian framework provides a powerful inferential technique for obtaining predictions from ordinary differential equation (ODE) models. Bayesian inference also manages information from experts or previous studies, and incorporates it in the estimation process. This results in a consistent approach for defining and propagating uncertainty within such ODE models taking into account the sources of uncertainty based on model structure and parameter values. We have determined the prior (or a priori) distributions of the parameters in our study based on values that can be found in the specific bibliography of related microbiological models. In a first step, the system of differential equations is discretized by means of a high order numerical Runge-Kutta method for non-stiff systems (Dormand & Prince, 1980). Then the discretized system is included in a probabilistic model and all parameters are estimated by means of a Markov Chain Monte Carlo method that is included in the program Stan for Bayesian model analysis (for full details of the procedure see Stan Development Team, 2014a).

We consider a Bayesian parameter estimation approach for computing the posterior distribution of parameters of the model in a similar way as described in Quinto, Marín, and Schaffner (2016). Briefly, we define reasonable prior distributions for the parameters μ_t and σ_t (Gelman et al., 2013). Quinto et al. (2016) collected the previous prior information about the centrality and variability of the parameters. A gamma prior distribution was taken for σ_t and a non-informative improper distribution (uniform over the real line) for μ_t parameters.

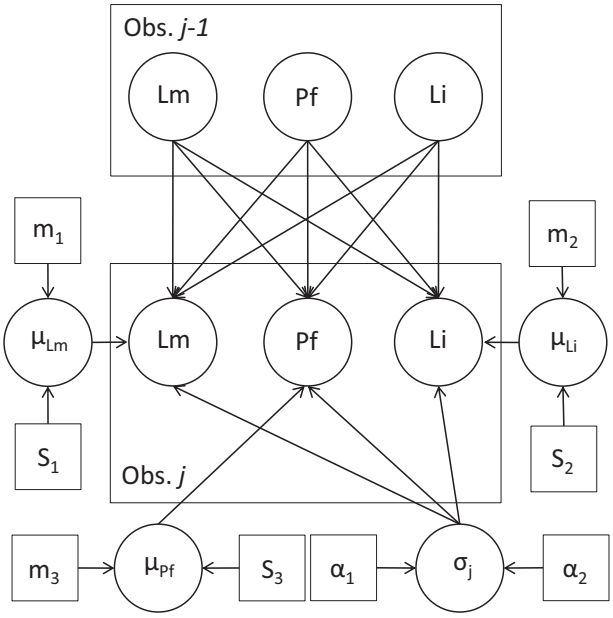

Fig. 1. Directed acyclic graph associated to the Bayesian model. Circles: random variables; squares: constants (initial parameters of the distributions of the variables); arrows: conditional dependence. Observed data (Obs. *j-1*) of *L. monocytogenes* (*Lm*), *L. innocua* (*Li*), and *P. fluorescens* (*Pf*) with their growth rates μ distributed with a Normal distribution of mean m and standard deviation S. The standard deviation of errors (σj) for every microorganism is distributed with a Gamma distribution with parameters α. The combinations of the microorganisms in the single cultures and in the co-cultures give the observed data.

Markov Chain Monte Carlo (MCMC) techniques can be applied to generate samples from the posterior distributions of parameters (Gilks, Richardson, & Spiegelhalter, 1996; Palacios, Marín, Quinto, & Wiper, 2014). In particular, the Hamiltonian Monte Carlo method (HMC), as described by Gelman et al. (2013), is implemented in the Stan software that was used to program the models here (Stan Development Team, 2014b). The algorithms were programmed in combination with R (R-Project, 2014) via Rstan (Stan Development Team, 2014b). All code is available from author JMM, upon request.

3. Results and discussion

3.1. Kinetic parameters

The maximum growth rate and the maximum population density of *L. monocytogenes*, *L. innocua*, and *P. fluorescens* in single cultures or co-cultured in SM are shown in Table 1. The R^2 and standard error of the fit values were acceptable ($R^2 > 0.90$). *L. monocytogenes* showed the lowest μ_{Lm} values when was co-cultured with *L. innocua* at 7 °C (0.86 d^{-1}), and with *L. innocua* or with *L. innocua* plus *P. fluorescens* at 14 °C (1.24 or 1.22 d^{-1}, respectively). The highest μ_{Lm} values were detected when it was co-cultured with *P. fluorescens* at 7 °C (0.99 d^{-1}) or 14 °C (1.48 d^{-1}). The maximum population density Lm_{max} was achieved when *L. monocytogenes* was single-cultured: 8.71 or 8.66 log CFU/ml at 7 and 14 °C, respectively. The Lm_{max} from co-cultures was observed when *L. monocytogenes* was mixed with *L. innocua*: 8.15 log CFU/ml at 4 °C and 7.88 log CFU/ml at 14 °C. The lowest Lm_{max} values were detected when *P. fluorescens* was present in the co-

Table 1
Starting concentrations (log CFU/ml), maximal growth rates (μ; d^{-1}), and maximal population densities (Lm_{max}, Li_{max}, and Pf_{max}; log CFU/ml) of *L. monocytogenes*, *L. innocua*, or *P. fluorescens* cultured alone or in co-culture in skimmed milk at 7 or 14 °C and estimated with DMFit software. Time to reach (*ttr*; d) a population density of 6 or 8 log CFU/ml.

Temp (°C)	Starting concentrations			Estimated parameters		Goodness of fit statistics		Time to reach (*ttr*) a population of	
	Lm	*Pf*	*Li*	μ_{Lm}	Lm_{max}	R^2	SE of fit	6 log CFU/ml	8 log CFU/ml
7	3.63	–	–	0.86	8.71	0.992	0.189	2.40	4.80
	3.41	3.93	–	0.99	6.80	0.989	0.147	2.40	–
	3.66	–	3.99	0.77	8.15	0.966	0.345	2.88	6.24
	3.09	4.18	3.69	0.93	6.65	0.968	0.282	2.72	–
14	3.68	–	–	1.34	8.66	0.978	0.326	1.60	3.40
	3.56	3.71	–	1.48	6.30	0.996	0.076	1.32	–
	3.28	–	3.38	1.24	7.88	0.985	0.230	1.60	3.40
	3.39	3.99	3.46	1.22	6.58	0.959	0.256	1.68	–
	Li	*Pf*	*Lm*	μ_{Li}	Li_{max}				
7	3.64	–	–	0.61	8.35	0.977	0.284	2.88	6.72
	3.66	4.25	–	0.83	6.56	0.968	0.206	2.24	–
	3.99	–	3.66	0.56	6.88	0.970	0.195	2.88	4.32[a]
	3.69	4.18	3.09	0.65	6.11	0.935	0.268	3.36	–
14	3.78	–	–	1.11	8.29	0.995	0.126	1.60	3.40
	3.58	3.98	–	1.86	6.14	0.923	0.282	1.08	–
	3.38	–	3.28	0.98	8.11	0.973	0.299	1.80	4.00
	3.46	3.99	3.39	1.66	5.76	0.943	0.299	1.20	–
	Pf	*Lm*	*Li*	μ_{Pf}	Pf_{max}				
7	3.82	–	–	1.10	8.80	0.943	0.470	1.12	3.08
	3.93	3.41	–	0.94	9.13	0.916	0.579	1.12	3.08
	4.25	–	3.66	0.95	8.99	0.921	0.518	0.84	3.08
	4.18	3.06	3.69	1.37	8.92	0.927	0.499	0.84	2.24
14	3.80	–	–	3.30	9.06	0.966	0.398	0.48	1.08
	3.71	3.56	–	3.52	9.08	0.952	0.472	0.48	1.08
	3.98	–	3.58	3.36	9.08	0.975	0.336	0.48	1.08
	3.99	3.39	3.5	3.58	9.08	0.980	0.301	0.48	1.08

[a] The culture achieved a population density of 7 log CFU/ml.

culture: 6.65 log CFU/ml at 7 °C, and 6.30 log CFU/ml at 14 °C.

The kinetic parameters of *L. innocua* showed similar behavior to those shown by *L. monocytogenes*. *L. innocua* showed the lowest μ_{Li} values when was co-cultured with *L. monocytogenes* (0.56 d^{-1} at 7 °C, and 0.98 d^{-1} at 14 °C), and the highest μ_{Li} values when it was co-cultured with *P. fluorescens* (0.83 d^{-1} at 7 °C, and 1.86 d^{-1} at 14 °C). At 7 °C the maximum population density Li_{max} was achieved when *L. innocua* was single-cultured (8.35 log CFU/ml) and in co-culture with *L. monocytogenes*: 6.88 log CFU/ml. At 14 °C the values of Li_{max} single-cultured or with *L. monocytogenes* were very similar: 8.29 and 8.11 log CFU/ml, respectively. The lowest Li_{max} values were detected when *P. fluorescens* plus *L. monocytogenes* were present in the co-culture: 6.11 and 5.76 log CFU/ml at 7 and 14 °C, respectively.

P. fluorescens showed the highest μ_{Pf} values when it was co-cultured with both species of *Listeria* at 7 °C (1.37 d^{-1}) and 14 °C (3.58 d^{-1}). The rest of the μ_{Pf} values were very similar among them at each temperature: 0.94–1.10 d^{-1} at 7 °C, and 3.30–3.52 d^{-1} at 14 °C. The maximum population density Pf_{max} was similar at both temperatures, with values ranging from 8.80 to 9.13 log CFU/ml.

Table 1 shows the time to reach (*ttr*) a population density of ca. 6 or 8 log CFU/ml; these populations were selected as (i) they typically occurred just before the maximum population densities were reached and (ii) both are part of the linear exponential growth phase within the growth rate shows a Log-Normal distribution independently of the inoculum and the environmental conditions (Métris, George, Peck, & Baranyi, 2003; Pin & Baranyi, 2006) (Table 1). At 7 °C all cultures arrived at a population of ca. 6 log CFU/ml with similar *ttr* values: 2.40–2.88 d for *L. monocytogenes* cultures, 2.24–3.36 d for *L. innocua* cultures, and 0.84–1.12 d for *P. fluorescens* cultures; similar results were found at 14 °C with lower *ttr* 6 log values indicating a faster growth: 1.32–1.68 d, 1.08–1.80 d, and 0.48 d, respectively. At both temperatures, the *ttr* 6 log values of *P. fluorescens* populations were lower than those from *L. monocytogenes* or *L. innocua* indicating a faster growth. Not all co-cultures reached a population density of ca. 8 log CFU/ml and the populations of *Listeria* spp. co-cultured with *P. fluorescens* did not exceed ~6–7 log CFU/ml at either temperature. The populations of *P. fluorescens* achieved lower *ttr* 8 log values than those from *L. monocytogenes* or *L. innocua* co-cultures, showing faster growth at both temperatures.

3.2. Logistic modeling of microbial interactions

Fig. 2 show representative logistic modeling of growth and decline periods for *L. monocytogenes* co-cultured with *L. innocua* and *P. fluorescens*, at 7 or 14 °C in skimmed milk. As is clear from the figure, logistic growth and decline models are adequate and fit the growth and decline data well.

Table 2 shows the estimated values of μ_{Lm}, μ_{Li}, and μ_{Lm}, and in two- or three-species co-cultures. *L. monocytogenes* showed the highest μ_{Lm} estimates at 7 °C co-cultured in the presence of *L. innocua* and *P. fluorescens* (0.764 d^{-1}), or co-cultured with *P. fluorescens* (0.706 d^{-1}); similar results were obtained at 14 °C: 1.638 d^{-1} for the *Lm(Li,Pf)* co-culture, and 1.546 d^{-1} for *Lm(Pf)*. The μ_{Li} estimates followed the same behavior than those of *L. monocytogenes* at both temperatures. The μ_{Pf} estimates were very similar from all the cultures at 7 °C (0.679–0.847 d^{-1}) or 14 °C (2.232–2.408 d^{-1}).

The estimated values of the decline rates *k* of *L. monocytogenes*, *L. innocua*, and *P. fluorescens* single-cultured, or in two- or three-species co-cultures are shown in Table 2. Decreasing populations were not detected for the three strains in single cultures at 7 °C, and for *L. monocytogenes* single-cultured at 14 °C. At 7 °C *L. monocytogenes* shows the highest decline rates k_{Lm} when was co-cultured in the presence of *L. innocua* plus *P. fluorescens* (− 0.272 d^{-1}), or co-cultured with *P. fluorescens* (− 0.226 d^{-1}); similar results were obtained at 14 °C: − 1.438 d^{-1} for *Lm(Li,Pf)*, and − 0.246 d^{-1} for *Lm(Pf)*. At 7 °C the k_{Li} estimates were detected only in the co-cultures *Li(Pf)* and *Lm(Li,Pf)*: − 0.096 and − 0.127 d^{-1}, respectively. At 14 °C the highest k_{Li} estimates were observed in the presence of *L. monocytogenes* plus *P. fluorescens* (− 0.291 d^{-1}) and co-cultured with *L. monocytogenes* (− 0.175 d^{-1}) or *P. fluorescens* (− 0.170 d^{-1}). The k_{Pf} estimates were

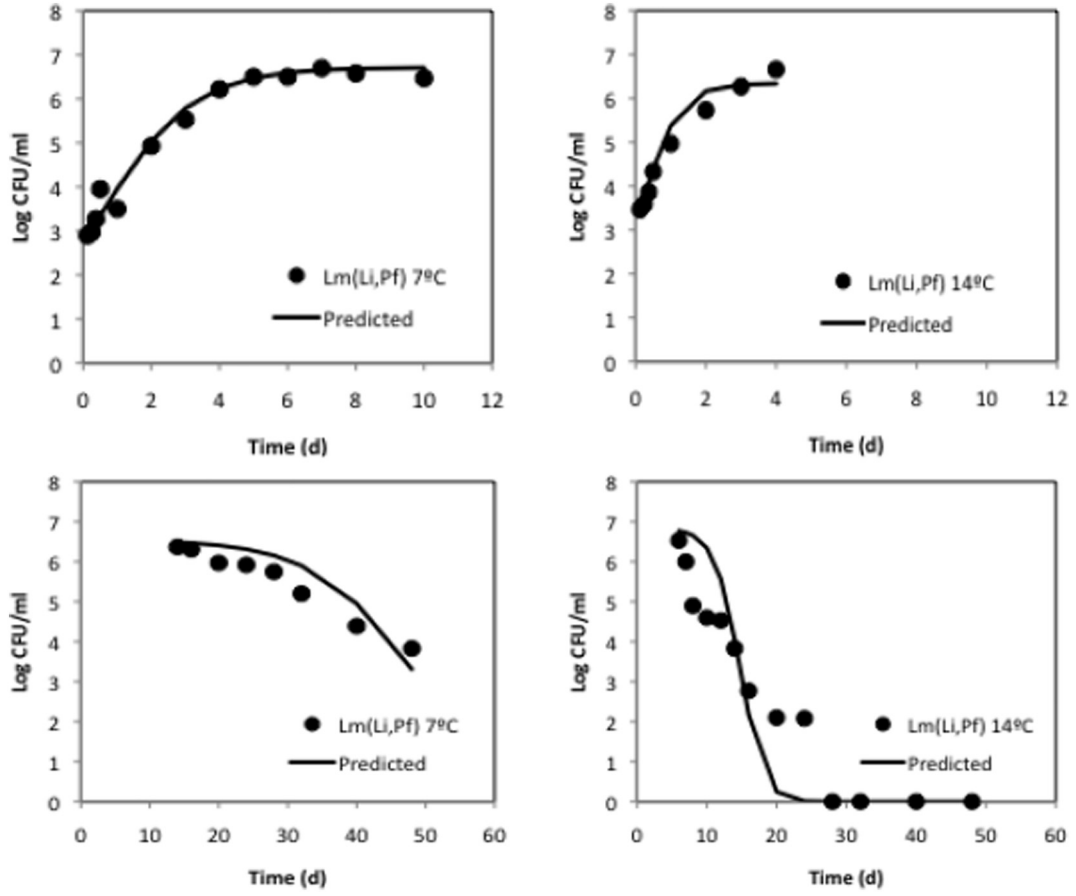

Fig. 2. Logistic modeling of growth and decline periods of *L. monocytogenes* co-cultured with *L. innocua* and *P. fluorescens*, *Lm(Li,Pf)*, at 7 or 14 °C in skimmed milk.

very similar from all the cultures at 7 °C (from − 0.014 to − 0.065 d^{-1}) or 14 °C (from − 0.026 to − 0.093 d^{-1}). Only in one case (k_{Li} from the *Lm(Li)* co-culture) the estimate is positive (0.097 d^{-1}), but the *p*-value is 0.072, > 0.05, and the hypothesis that it is not significantly different than zero is accepted.

3.3. Bayesian modeling of microbial interactions

Fig. 3 shows representative examples of the Bayesian inference of growth and decline periods for *L. monocytogenes* co-cultured with *L. innocua* and *P. fluorescens*, at 7 or 14 °C in skimmed milk. Additional examples are shown in Supplement Material 1.1–1.5. The limits of the respective Highest Posterior Density (HPD) 95% intervals (2.5 and 97.5%) are shown in Fig. 3 and Table 3. The growth models are suitable with close HPD intervals. Interestingly, the HPD decline intervals at 14 °C are relatively close, except for *P. fluorescens* co-cultured with *L. monocytogenes* (Supplement Material 1.2); a similar phenomenon was observed for *L. monocytogenes* or *L. innocua* in their 3-strains co-cultures at 7 °C (Supplement Material 1.5). Bayesian inference model yields wide confidence intervals under certain circumstances. Those circumstances all share a certain attribute, namely that the decline data do not show a continuous downward trend, but instead show an increase or recovery of the population. This typically occurs in the last 2–3 points of the curve. We believe that the solution here would be to collect more observations in the declining part of the curve so that those final few points play a less important role in the model fitting. In order to probe this theory, missing time values between the sampling times were replaced by linear interpolation, which resulted in better fits of the decline period (Supplement Material 1.6–1.7).

Table 3 shows posterior means and medians (50%) of parameters and the limits of the respective HPD intervals (2.5% and 97.5%) of growth (μ_{Lm}, μ_{Li}, or μ_{Pf}) and decline (k_{Lm}, k_{Li}, or k_{Pf}) rates of *L. monocytogenes*, *L. innocua*, and *P. fluorescens* in single cultures or in two- or three-species co-cultures. The estimated values followed similar trends as those obtained with the deterministic model (Table 2), although the values were slightly lower. *L. monocytogenes* shows the highest μ_{Lm} estimates at 7 °C when co-cultured in the presence of *L. innocua* plus *P. fluorescens* (0.684 d^{-1}), or co-cultured with *P. fluorescens* (0.616 d^{-1}); similar results were obtained at 14 °C: 1.317 d^{-1} for the *Lm(Li,Pf)* co-culture, and 1.048 d^{-1} for *Lm(Pf)*. The μ_{Li} estimates follow the same behavior than those of *L. monocytogenes* at both temperatures. The μ_{Pf} estimates were very similar from all the cultures at 7 °C (0.644–0.835 d^{-1}) or 14 °C (1.845–1.695 d^{-1}).

Decreasing populations were not detected for the three strains in single cultures at 7 °C, neither for *L. monocytogenes* single-cultured at 14 °C. Most of the values of *k* were negative but some of them positive; it is important to note that those positive values were into a HPD interval between a negative 25% interval value and a positive 97.5% interval value, given the zero value into the interval. This means that the estimated values are not significantly different from zero (indicating neither growth nor decline of the microorganisms) with a 97.5% of confidence; the populations would remain stable. At 7 °C *L. monocytogenes* shows the highest decline rates k_{Lm} when was co-cultured in the presence of *P. fluorescens* (− 0.157 d^{-1}) or with *L. innocua* plus *P. fluorescens* (− 0.099 d^{-1}); at 14 °C the fastest decline rates were observed from *Lm(Li,Pf)* and *Lm(Pf)* co-cultures: − 0.764 d^{-1} and − 0.302 d^{-1}, respectively. At 7 °C all the k_{Li} estimates were positive, but within negative-positive HPD intervals; at 14 °C the highest k_{Li} estimates were observed in the presence of *L. monocytogenes* plus *P. fluorescens* (− 0.275 d^{-1}) and co-cultured with *P. fluorescens* (− 0.108 d^{-1}). The k_{Pf} estimates varied from positive to negative values due to the randomness of the errors. At 7 °C the highest decline rate

Table 2
Logistic estimates and statistic values of the maximal exponential growth rates μ and decline rates k of *L. monocytogenes*, *L. innocua*, and *P. fluorescens* cultured alone (*Lm*, *Li*, *Pf*), or co-cultured in 2-especies (*Lm + Pf*, *Li + Pf*, *Lm + Li*) or 3-especies co-cultures (*Lm + Li + Pf*) at 7 or 14 °C in skimmed milk.

Cultures	Temp (°C)	μ/k	Estimate	Std. Error	t value	Pr (> |t|)	RSE
Growth:							
Lm, Li, Pf	7	μ_{Lm}	0.482	0.019	26.01	< 2e − 16	0.204
		μ_{Li}	0.338	0.017	19.88	4.22e − 15	0.277
		μ_{Pf}	0.679	0.081	8.355	4.07e − 8	0.572
	14	μ_{Lm}	0.676	0.060	11.36	1.98e − 10	0.384
		μ_{Li}	0.676	0.033	20.52	2.75e − 11	0.197
		μ_{Pf}	2.232	0.255	8.757	2.74e − 6	0.417
Lm + Pf	7	μ_{Lm}	0.706	0.064	11.03	1.98e − 10	0.406
		μ_{Pf}	0.847	0.070	12.08	3.49e − 11	
	14	μ_{Lm}	1.546	0.198	7.792	1.86e − 6	0.454
		μ_{Pf}	2.408	0.267	9.039	3.22e − 7	
Li + Pf	7	μ_{Li}	0.609	0.061	9.903	1.44e − 9	0.427
		μ_{Pf}	0.748	0.067	11.125	1.68e − 10	
	14	μ_{Li}	1.617	0.125	12.89	7.24e − 10	0.265
		μ_{Pf}	2.371	0.165	14.40	1.41e − 10	
Lm + Li	7	μ_{Lm}	0.448	0.015	29.4	< 2e − 16	0.201
		μ_{Li}	0.320	0.012	26.7	< 2e − 16	
	14	μ_{Lm}	0.886	0.060	14.85	8.85e − 11	0.307
		μ_{Li}	0.824	0.057	14.39	1.42e − 10	
Lm + Li + Pf	7	μ_{Lm}	0.764	0.054	14.07	< 2.0e − 16	0.372
		μ_{Li}	0.559	0.044	12.76	1.70e − 15	
		μ_{Pf}	0.770	0.052	14.87	< 2.0e − 16	
	14	μ_{Lm}	1.638	0.122	13.34	1.01e − 11	0.276
		μ_{Li}	1.481	0.116	12.76	2.34e − 11	
		μ_{Pf}	2.244	0.145	15.46	6.01e − 13	
Decline:							
Lm, Li, Pf	14	k_{Li}	− 0.104	0.004	− 24.2	3.27e − 7	0.288
		k_{Pf}	− 0.093	0.005	− 18.07	9.02e − 8	0.352
Lm + Pf	7	k_{Lm}	− 0.226	0.012	− 19.42	1.51e − 12	0.318
		k_{Pf}	− 0.014	0.007	− 2.03	0.0599 c	
	14	k_{Lm}	− 0.246	0.040	− 6.141	2.41e − 6	1.036
		k_{Pf}	− 0.046	0.020	− 2.351	0.0273 b	
Li + Pf	7	k_{Li}	− 0.096	0.009	− 10.258	1.92e − 8	0.188
		k_{Pf}	− 0.033	0.007	− 4.769	0.0002	
	14	k_{Li}	− 0.170	0.032	− 5.302	2.54e − 5	0.814
		k_{Pf}	− 0.092	0.017	− 5.396	2.03e − 5	
Lm + Li	7	k_{Lm}	− 0.175	0.020	− 8.933	8.68e − 4	0.121
		k_{Li}	0.097	0.040	2.427	0.072 c	
	14	k_{Lm}	− 0.008	0.012	− 0.634	0.535 d	0.323
		k_{Li}	− 0.175	0.012	− 15.081	7.05e − 11	
Lm + Li + Pf	7	k_{Lm}	− 0.272	0.032	8.398	1.22e − 7	0.383
		k_{Li}	− 0.127	0.032	3.952	0.0009	
		k_{Pf}	− 0.065	0.018	3.599	0.0021 a	
	14	k_{Lm}	− 1.438	0.122	11.775	6.65e − 14	0.777
		k_{Li}	− 0.291	0.055	5.317	5.69e − 6	
		k_{Pf}	− 0.026	0.004	6.125	4.74e − 7	

RSE, residual standard error; Significance code: 0.001 “a”; 0.01 “b”; 0.05 “c”; 0.1 “d”.

was detected from *Lm(Pf)* co-culture ($-0.012\ d^{-1}$). At 14 °C most of the decline rates were similar and negative (from -0.025 to $-0.068\ d^{-1}$) with only one positive value ($0.047\ d^{-1}$ from *Lm(Pf)* co-cultures).

Table 4 shows posterior means and medians (50%) of parameters and the limits of the respective HPD intervals (2.5% and 97.5%) of the standard deviations (σ) of the growth (σ_{Lm}, σ_{Li}, or σ_{Pf}) and decline (σ'_{Lm}, σ'_{Li}, or σ'_{Pf}) rates of *L. monocytogenes*, *L. innocua*, and *P. fluorescens* in single cultures or in two- or three-species co-cultures. The standard deviation can be interpreted as the random error associated with the real observations of the predicted concentration of the microorganisms. The posterior means of the standard deviations are shown in Table 4.

3.4. Discussion

The presence of *L. monocytogenes* in refrigerated food, including milk and milk products, represents a potential risk for consumers, particularly for those who are immunocompromised. Antagonistic microorganisms, like *Pseudomonas* spp., may be useful in controlling the growth of *L. monocytogenes*. According to Farrag and Marth (1989a, 1989b), *P. fluorescens* and *P. aeruginosa* have a moderate negative effect on growth of *L. monocytogenes* Scott A at 13 °C in TSB or skimmed milk. Similar inhibitory results were found by Gram (1993) when siderophores-producing strains of *Pseudomonas* spp., mainly *P. aeruginosa*, were co-cultured with *L. monocytogenes* strains. Farrag and Marth (1989a, 1989b) found final counts of ca. 6–7 log CFU/ml of *L. monocytogenes* co-cultured with *P. fluorescens* at 13 °C; similar results were found in our study at 14 °C (6 log CFU/ml) with a time-to-reach that population (*ttr* 6 log) of 1–2 d. At 7 °C they found that populations of *L. monocytogenes* decreased in the presence of *P. fluorescens* with final counts of ca. 7–8 log CFU/ml; we found lower final counts at the same temperature: 6–7 CFU/ml with a *ttr* 6 log of 2–3 d. These authors did not find significant changes in pH between the beginning and the end of the incubation period (60 d) of the co-cultures; in our study pH values of the microorganisms in single cultures or in co-culture (data not shown) decreased from 6.7–6.8 at time zero to 4.2–4.6 at the end of the experiment at both temperatures. Lebert, Robles-Olvera, and Lebert (2000) found that *Pseudomonas fragi* and *P. fluorescens* did not affect the growth of *L. monocytogenes* and *L. innocua* on decontaminated meat at 6 °C; however, in naturally contaminated meat inoculated with *L.*

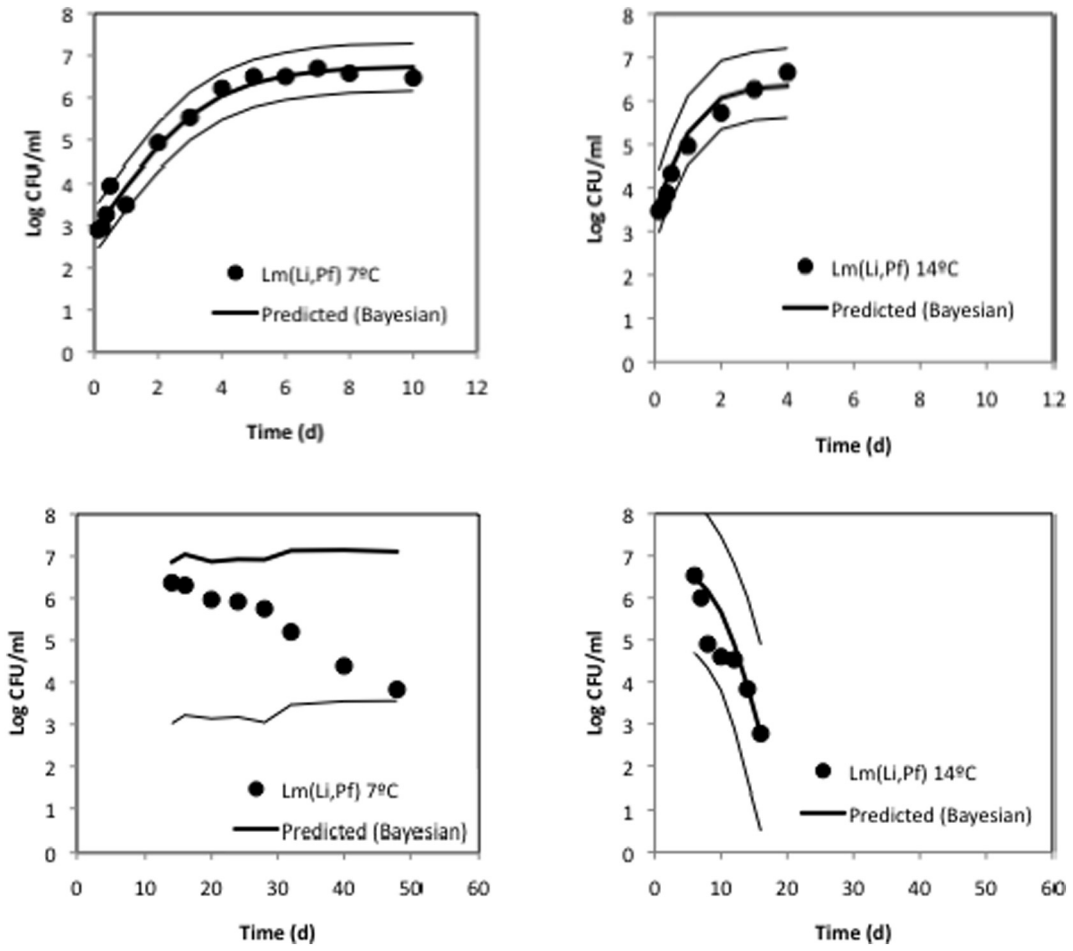

Fig. 3. Bayesian inference of growth and decline periods of *L. monocytogenes* co-cultured with *L. innocua* and *P. fluorescens*, *Lm(Li,Pf)*, at 7 or 14 °C in skimmed milk. Highest Posterior Density (HPD) 95% intervals (2.5 and 97.5%) are shown.

innocua, it did not grow until *Pseudomonas* reached the stationary phase. These differences in the results between *L. monocytogenes* and *L. innocua* co-cultured with *P. fluorescens* contrast with ours, as we found similar inhibitory effects of *P. fluorescens* on *L. innocua* or *L. monocytogenes*.

Some authors reported that when competitors can grow faster than *L. monocytogenes*, as found for *L. innocua* (Besse, Audinet, Kérouanton, Colin, & Kalmokoff, 2005; Beumer, Giffel, Anthonie, & Cox, 1996; Curiale & Lewus, 1994; MacDonald & Sutherland, 1994; Oravcova, Trncikova, Kuchta, & Kaclikova, 2008), the counts of *L. monocytogenes* could be lower. However, and similarly to Cornu, Kalmokoff, and Flandrois (2002) and Besse et al. (2010), we found that *L. innocua* followed similar behavior than *L. monocytogenes* at 7 and 14 °C, with differences of 1 logarithmic cycle in the N_{max} in some cases. At both temperatures the single cultures of *L. innocua* reached 8 log CFU/ml, and the single cultures of *L. monocytogenes* grew up to 9 log CFU/ml. When *L. monocytogenes* was present, the counts of *L. innocua* (cultures *Li (Lm)*) reached 7 or 8 log CFU/ml at 7 or 14 °C, and the counts of *L. monocytogenes* (cultures *Lm(Li)*) reached 8 log CFU/ml at both temperatures. When *P. fluorescens* was present in two- or three-species cultures, *L. innocua* or *L. monocytogenes* grew up to a N_{max} of 6–7 log CFU/ml at both temperatures. The difference between both temperatures was the *ttr* the N_{max}; the fastest cultures of *L. innocua* were those in single cultures at 14 °C (Table 1).

P. fluorescens grew faster (higher μ_{Pf} values than *Listeria* species) to higher N_{max} in single cultures as well as in co-culture at 7 and 14 °C (Table 1). This behavior was not detected in the *Listeria* species; they showed clear differences when they were co-cultured in the presence of *P. fluorescens*: lower N_{max}; however, μ_{Lm} or μ_{Li} values seem not be affected by the presence of *P. fluorescens*. Similar behaviors were found in previous studies (Quinto et al., 2016) when *L. monocytogenes* was co-cultured with *Lactobacillus sakei*. The increase in growth rate of a microorganism in co-culture has been observed often in systems with interspecific competition. The reasons for this phenomenon are diverse (Hibbing, Fuqua, Parsek, & Peterson, 2010) and under study. Trejo-Hernández, Andrade-Domínguez, Hernández, and Encarnación (2014) reported that mixed biofilms of *Pseudomonas aeruginosa* and *Candida albicans* showed differential expression between species of virulence proteins, multidrug resistance-associated proteins, proteases and cell defence, stress and iron-regulated proteins. Furthermore, both species displayed an increase in mutability. Pekkonen, Ketola, and Laakso (2013) observed different behaviors working with ancestral and evolved bacterial clones; indeed, evolved clones of *Serratia marcescens* co-cultured with *Novosophingobium capsulatum* had higher survival and slower growth rate than their ancestor.

Buchanan and Bagi (1999) reported that when *P. fluorescens* influenced the growth of *L. monocytogenes*, the primary effect was a suppression of the maximal population density reached by the pathogen generally associated with low incubation temperatures (4 °C); slight increases (< 1 log CFU/ml) in the maximum population density attained by *L. monocytogenes* were observed when it was grown in the presence of *P. fluorescens* at higher temperatures (12 and 19 °C). This is consistent with the Jameson Effect (Jameson, 1962) with regard to the suppression phenomenon, which is attributed to the production of specific inhibitors of growth by one species, which has reached the stationary phase against another, which has not (Jameson, 1962;

Table 3
Bayesian estimates of the posterior mean and Highest Posterior Density (HPD) intervals (95%: 2.5 and 97.5%, and the 50%) of the growth (μ) and decline (k) rates of *L. monocytogenes*, *L. innocua*, and *P. fluorescens* cultured alone (*Lm*, *Li*, *Pf*), or co-cultured in 2-especies (*Lm* + *Pf*, *Li* + *Pf*, *Lm* + *Li*) or 3-especies co-cultures (*Lm* + *Li* + *Pf*) at 7 or 14 °C in skimmed milk.

Cultures	Temp	μ/k	Mean	HPD Intervals		
	(°C)			2.5%	50%	97.5%
Growth:						
Lm, Li, Pf	7	μ_{Lm}	0.466	0.438	0.465	0.497
		μ_{Li}	0.331	0.300	0.330	0.364
		μ_{Pf}	0.672	0.516	0.661	0.895
	14	μ_{Lm}	0.643	0.542	0.640	0.766
		μ_{Li}	0.639	0.589	0.638	0.693
		μ_{Pf}	1.736	1.461	1.732	2.028
Lm + Pf	7	μ_{Lm}	0.616	0.560	0.614	0.686
		μ_{Pf}	0.737	0.650	0.734	0.838
	14	μ_{Lm}	1.048	0.854	1.033	1.322
		μ_{Pf}	1.695	1.368	1.685	2.084
Li + Pf	7	μ_{Li}	0.665	0.576	0.662	0.770
		μ_{Pf}	0.835	0.706	0.831	0.990
	14	μ_{Li}	1.239	1.103	1.239	1.377
		μ_{Pf}	1.845	1.674	1.845	2.020
Lm + Li	7	μ_{Lm}	0.435	0.401	0.431	0.498
		μ_{Li}	0.310	0.284	0.309	0.345
	14	μ_{Lm}	0.711	0.638	0.711	0.789
		μ_{Li}	0.706	0.620	0.704	0.804
Lm + Li + Pf	7	μ_{Lm}	0.684	0.610	0.682	0.763
		μ_{Li}	0.459	0.413	0.458	0.513
		μ_{Pf}	0.644	0.558	0.643	0.734
	14	μ_{Lm}	1.317	1.091	1.309	1.587
		μ_{Li}	1.163	0.966	1.168	1.330
		μ_{Pf}	1.802	1.512	1.809	2.057
Decline:						
Lm, Li, Pf	14	k_{Li}	− 0.049	− 0.072	− 0.066	− 0.048
		k_{Pf}	− 0.068	− 0.074	− 0.069	− 0.062
Lm + Pf	7	k_{Lm}	− 0.157	− 0.168	− 0.157	− 0.146
		k_{Pf}	− 0.012	− 0.019	− 0.012	− 0.005
	14	k_{Lm}	− 0.302	− 0.963	− 0.170	− 0.091
		k_{Pf}	0.047	− 0.067	− 0.041	0.462
Li + Pf	7	k_{Li}	0.193	− 0.115	− 0.061	1.845
		k_{Pf}	0.142	− 0.189	− 0.021	1.598
	14	k_{Li}	− 0.108	− 0.135	− 0.108	− 0.082
		k_{Pf}	− 0.062	− 0.079	− 0.062	− 0.043
Lm + Li	7	k_{Lm}	0.580	− 0.475	0.489	2.040
		k_{Li}	0.916	− 0.044	0.839	2.305
	14	k_{Lm}	− 0.034	− 0.048	− 0.035	− 0.020
		k_{Li}	− 0.099	− 0.121	− 0.099	− 0.076
Lm + Li + Pf	7	k_{Lm}	− 0.099	− 1.227	− 0.372	1.788
		k_{Li}	0.832	− 0.459	0.700	2.313
		k_{Pf}	0.310	− 0.580	0.147	1.761
	14	k_{Lm}	− 0.764	− 0.945	− 0.762	− 0.598
		k_{Li}	− 0.275	− 0.354	− 0.272	− 0.211
		k_{Pf}	− 0.025	− 0.031	− 0.025	− 0.020

Table 4
Bayesian estimates of the posterior mean, the 95% (2.5 and 97.5%) and the 50% intervals of the standard deviations for growth (σ) and decline ($-\sigma$) periods of *L. monocytogenes*, *L. innocua*, and *P. fluorescens* cultured alone (*Lm*, *Li*, *Pf*), or co-cultured in 2-especies (*Lm* + *Pf*, *Li* + *Pf*, *Lm* + *Li*) or 3-especies co-cultures (*Lm* + *Li* + *Pf*) at 7 or 14 °C in skimmed milk.

Cultures	Temp	σ	Mean	HPD Intervals		
	(°C)			2.5%	50%	97.5%
Growth:						
Lm, Li, Pf	7	σ_{Lm}	0.173	0.128	0.170	0.237
		σ_{Li}	0.265	0.196	0.260	0.366
		σ_{Pf}	0.552	0.409	0.540	0.759
	14	σ_{Lm}	0.422	0.312	0.414	0.579
		σ_{Li}	0.171	0.116	0.165	0.259
		σ_{Pf}	0.314	0.204	0.302	0.498
Lm + Pf	7	σ_{Lm}	0.155	0.098	0.148	0.253
		σ_{Pf}	0.520	0.342	0.500	0.807
	14	σ_{Lm}	0.250	0.144	0.232	0.459
		σ_{Pf}	0.500	0.285	0.475	0.867
Li + Pf	7	σ_{Li}	0.184	0.118	0.175	0.296
		σ_{Pf}	0.492	0.327	0.472	0.768
	14	σ_{Li}	0.228	0.138	0.215	0.390
		σ_{Pf}	0.187	0.109	0.176	0.329
Lm + Li	7	σ_{Lm}	0.204	0.135	0.192	0.353
		σ_{Li}	0.215	0.134	0.210	0.320
	14	σ_{Lm}	0.242	0.150	0.232	0.396
		σ_{Li}	0.419	0.265	0.402	0.666
Lm + Li + Pf	7	σ_{Lm}	0.261	0.172	0.251	0.407
		σ_{Li}	0.171	0.108	0.164	0.274
		σ_{Pf}	0.581	0.385	0.558	0.899
	14	σ_{Lm}	0.338	0.173	0.312	0.649
		σ_{Li}	0.201	0.108	0.186	0.383
		σ_{Pf}	0.326	0.176	0.302	0.618
Decline:						
Lm, Li, Pf	14	$-\sigma_{Li}$	0.384	0.205	0.334	0.969
		$-\sigma_{Pf}$	0.282	0.170	0.266	0.489
Lm + Pf	7	$-\sigma_{Lm}$	0.236	0.142	0.222	0.412
		$-\sigma_{Pf}$	0.203	0.120	0.191	0.355
	14	$-\sigma_{Lm}$	0.768	0.136	0.811	1.437
		$-\sigma_{Pf}$	0.911	0.340	0.546	2.964
Li + Pf	7	$-\sigma_{Li}$	0.396	0.147	0.275	1.019
		$-\sigma_{Pf}$	0.352	0.180	0.316	0.726
	14	$-\sigma_{Li}$	0.466	0.294	0.445	0.756
		$-\sigma_{Pf}$	0.676	0.433	0.647	1.090
Lm + Li	7	$-\sigma_{Lm}$	0.796	0.300	0.698	1.859
		σ_{Li}	0.289	0.088	0.217	0.918
	14	$-\sigma_{Lm}$	0.385	0.208	0.352	0.753
		$-\sigma_{Li}$	0.546	0.303	0.507	1.030
Lm + Li + Pf	7	$-\sigma_{Lm}$	1.793	0.871	1.689	3.264
		$-\sigma_{Li}$	0.615	0.090	0.582	1.513
		$-\sigma_{Pf}$	0.477	0.237	0.419	1.153
	14	$-\sigma_{Lm}$	0.879	0.595	0.846	1.353
		$-\sigma_{Li}$	0.473	0.314	0.454	0.739
		$-\sigma_{Pf}$	0.484	0.316	0.467	0.746

Mellefont et al., 2008). In contrast to Buchanan and Bagi (1999), we did not find differences between low and high temperatures; indeed, neither *L. monocytogenes* nor *L. innocua* did reach a maximal population density of 8 log CFU/ml at 7 or 14 °C in the presence of *P. fluorescens* but counts of 6 log CFU/ml did at both temperatures. We obtained different results in the time to reach that population density (i.e. 6 log CFU/ml), founding *ttr* 6 log values varying ca. 24 h between 7 and 14 °C. Mellefont et al. (2008) examined the effect of *P. fluorescens* on growth of *L. monocytogenes* in non-selective medium co-cultures, concluding that the initial inoculum concentrations governed which species dominates suppressing growth of the others. Previously, Petran and Swanson (1993) suggested that high densities of *L. innocua* might impede the growth of *L. monocytogenes*. According to Besse et al. (2010), the overgrowth of *L. monocytogenes* by *L. innocua* resulted from interactions in late exponential phase, where growth of both strains stopped when the dominant strain reached its maximum population density. When the faster growing *L. innocua* reached stationary phase in mixed cultures in Fraser broths at 30 or 37 °C, growth of *L. monocytogenes* also stopped. These authors suggested that the overgrowth could partially be explained in terms of a nutritional competition. It could also be explained in terms of quorum sensing stimuli (Diggle, Griffin, Campbell, & West, 2007; West, Griffin, Gardner, & Diggle, 2006); once the faster microorganism reaches its maximum population density the concentration of signaling molecules also reaches its maximum, indicating to the second species of the mixed culture that the maximum total population density of the culture (or carrying capacity of the system) has been achieved, finishing its growth.

Al-Zeyara et al. (2011) identified the ability of low numbers of *L. monocytogenes* to achieve high cell concentrations in enrichment media being highly dependent on the total numbers and types of competing microbes initially present. These authors investigated the ability of *L. monocytogenes* to compete with the natural microflora of different foods, finding that an inoculum of 0.1 CFU/ml reached concentrations above 3 log CFU/ml when the competitors' initial number was below

3 log CFU/ml; competitors' populations above 4.5 log CFU/ml inhibited the growth of *L. monocytogenes* depending on the food, being the microflora of beef mince, salami and goat cheese particularly inhibitory. Quinto et al. (2016) reported that interspecies competition between *Lactobacillus sakei* MN and *L. monocytogenes* changes with temperature and starting concentration of both microorganisms. In the present study, the initial concentration of both microorganisms was not considered as a variable and this may merit further investigations.

A better understanding of the behavior of foodborne pathogens in the presence of the natural microflora of foods is relevant not only to the development of improved detection but also to improving predictive models of pathogen growth in food and risk assessments (Powell, Schlosser, & Ebel, 2004). Oravcova et al. (2008) noted that the overgrowth of *L. monocytogenes* by a non-pathogenic species of *Listeria* can mask the presence of low numbers of the pathogenic strain in the food sample, increasing the possible number of false negatives. Al-Zeyara et al. (2011) reported that *Pseudomonas* spp. showed an inhibition effect on *L. monocytogenes* co-cultured during enrichment of a range of different foods including dairy products, in TSB, Half Fraser Broth, or ONE Broth at 30 °C for 24–48 h.

The progress of a *Listeria* population is dependent on a complex set of interactions (Besse et al., 2010): the production of inhibitors (Cornu et al., 2002; Quinto et al., 2016; Schillinger, Kaya, & Lucke, 1991; Winkowski & Montville, 1992), competition from the background flora (Al-Zeyara et al., 2011; Dallas, Tran, Poindexter, & Hitchins, 1991), differences in growth rates among the different *Listeria* species (Beumer et al., 1996; Cornu et al., 2002; Curiale & Lewus, 1994; Duh & Schaffner, 1993; MacDonald & Sutherland, 1994) and interactions with the food matrix (Al-Zeyara et al., 2011; Besse et al., 2005; Santillan, D'Aquino, & Franco, 1997; Schillinger et al., 1991). Bacteria often live within matrix-embedded communities, termed biofilms, which are now understood to be a major mode of microbial life (Nadell et al., 2016). *P. fluorescens* produces extracellular matrix materials that give secreting cells a positional advantage over competitors (Nadell et al., 2016). Competition for limited resources can be achieved through the production of acids, bacteriocins or other metabolites. When Mellefont et al. (2008) used a non-producing bacteriocin strain of *Lactobacillus plantarum* co-cultured with *L. monocytogenes*, they observed a non-specific inhibition based on the exploitation of nutrients for achieving the maximum population density. Buchanan and Bagi (1997) found similar results when both producing and non-producing bacteriocin strains of *Carnobacterium piscicola* were co-cultured with *L. monocytogenes*. Quinto et al. (2016) found that the primary effect of a bacteriocin-producing strain of *Lactobacillus sakei* on *L. monocytogenes* was the suppression of its maximum population density. Those results suggest either a depletion of a critical nutrient or the production of an inhibitory extracellular agent associated with the microorganism reaching a specific population density. Bacterial members of a culture may exchange information to synchronize their behavior, a phenomenon called quorum sensing. Both Gram-positive and -negative bacteria communicate within and between species, monitoring population density and modulating gene expression (Dubey & Ben-Yehuda, 2011; Ng & Bassler, 2009). This phenomenon could explain the fact that the inhibitory effect of some bacteria is limited to the maximal population density of the inhibited microorganism, because of the inhibitor would need to reach the necessary population density to express its inhibitory effect.

3.5. Conclusions

The aim of this work was to examine the nature of the competition between *L. monocytogenes*, *L. innocua* and *Pseudomonas fluorescens* (a psychrotrophic Gram-negative spoilage strain present in milk and milk products) in two- or three-species co-cultures in skimmed milk at 7 and 14 °C, and to model those interactions. We have used a parametric Bayesian approach in this work. The parametric Bayesian approach assumes that parameters (μ's and σ) are also random variables with known prior distributions. The inference consists of estimating the conditional distribution of the parameters given the observed data and the prior distributions. The most widely used approach is based on Monte Carlo Markov Chain (MCMC) methods. These methods are computationally quite efficient and they permit the introduction of relevant information from other sources or experts. This can be a clear advantage for improving the predictive ability of the models. On the other hand, significance concepts based on *p*-values are not relevant in this context, as all findings are based on a pure probabilistic background, including confidence intervals that are computed as rigorous credibility intervals without a critical dependence of the sample size. We found that the highest *Listeria* populations were achieved by one- or two-species co-cultures at both temperatures, decreasing in co-culture with *P. fluorescens*. *Listeria* strains reached a maximal population den-sity of ~6–7 log CFU/ml at 7 or 14 °C in the presence of *P. fluorescens*, with times to reach a 6 log population occurring within 24 h at tem-peratures of 7 and 14 °C. The populations of *P. fluorescens* achieved a maximum population density of 9 log CFU/ml, with lower time-to-reach a population of 8 log CFU/ml values than when co-cultured with *L. monocytogenes* or *L. innocua*, showing a faster growth at both temperatures. The results obtained in our work also show that there is not a direct correlation between the growth rate of *P. fluorescens* and its inhibitory effect on *Listeria* species. The use of some species from the natural food microflora to inhibit pathogen growth may be an important tool to enhance the safety of refrigerated foods such as milk and dairy products, but our results show that the interactions between two or three organisms in a model food system are more complex than previously thought and require more study.

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