Modelling Growth and Decline in a Two-Species Model System: Pathogenic Escherichia coli O157:H7 and Psychrotrophic Spoilage Bacteria in Milk

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Abstract: Shiga toxin-producing Escherichia coli O157:H7 is a food-borne pathogen and the major cause of hemorrhagic colitis. Pseudomonas is the genus most frequent psychrotrophic spoilage microorganisms present in milk. Two-species bacterial systems with E. coli O157:H7, non-pathogenic E. coli, and P. fluorescens in skimmed milk at 7, 13, 19, or 25 °C were studied. Bacterial interactions were modelled after applying a Bayesian approach. No direct correlation between P. fluorescens’s growth rate and its effect on the maximum population densities of E. coli species was found. The results show the complexity of the interactions between two species in a food model. The use of natural microbiota members to control foodborne pathogens could be useful to improve food safety during the processing and storage of refrigerated foods.

Keywords: Shiga toxin-producing Escherichia coli O157:H7; Pseudomonas fluorescens; co-culture; competition; Bayesian modeling

1. Introduction

Shiga toxin-producing Escherichia coli O157:H7 strains are foodborne pathogens causing hemorrhagic colitis or the hemolytic uremic syndrome [1]. These microorganisms can be transmitted through consumption of undercooked meat, vegetables, contaminated water, unpasteurized dairy products, and raw milk [2–5]. The survival capacity of E. coli O157:H7 can go as far as several days or weeks in milk and dairy products [6–8] showing the importance of post processing contamination and the associated health risks.

Soil, water and vegetation are the main sources of psychrotrophic spoilage microorganisms, such as Pseudomonas, to milk [9–11]. During the pre-processing period (3–4 days) prior to pasteurization, psychrotrophic bacteria can grow and cause significant chemical changes [10,12]. Pseudomonas also appears in pasteurized dairy products as a post-processing contaminant [9,13].

Different bacterial species interact without physical barriers in many natural environments, and foods are one such example where co-culture experiments have shown the prevailing genotypes in mixed cultures [14–17]. Spoilage microorganisms are able to enhance, limit or be neutral on the growth of pathogenic species [18]. P. fluorescens produces extracellular materials resulting in a competitive advantage over other species [17] such that the competitor is physically displaced [19] or the nutrients access impeded [20]. In early research on the topic [21,22], enhancement of
Staphylococcus aureus’s growth in the presence of Pseudomonas spp was reported. Marshall and Schmidt [22,23] and Farrag and Marth [12] found similar results when Listeria monocytogenes was co-cultured in the presence of P. fluorescens. Other studies reported conversely that P. fluorescens can inhibit the growth of L. monocytogenes [18,24–30]. The specific addition of glucose to TSB broth stimulated the inhibition of E. coli O157:H7 by P. fluorescens [31]. Liao [32] and Liao et al. [33] reported that P. fluorescens and Bacillus spp. were able to act as biocontrol agents of Salmonella Saintpaul on Jalapeno pepper or of E. coli O157:H7 on TSA agar and bell pepper disks. On the surface of spinach leaves, Olaya et al. [34] reported that P. fluorescens moderately suppressed the growth of E. coli O157:H7. The same research group also showed P. fluorescens inhibition of E. coli O157:H7 in poor environments such as distilled water or buffered peptone water [35].

The aim of our work is to study the interaction between co-culturing bacterial species using Bayesian inference. The Bayesian approach provides a consistent framework for estimating parameters from a model using prior knowledge about the system to improve the estimations [36], with the advantage of including uncertainties within the model [37]. Two-species systems were co-cultured with E. coli O157:H7 or non-pathogenic E. coli and P. fluorescens in skimmed milk over a range of temperatures and times that are both typical and atypical for milk storage and distribution.

2. Materials and Methods

2.1. Bacterial Cultures and Inoculation

Three strains of Escherichia coli: O157:H7 LCDC 86-51 (EcO1; Shiga toxin-producing strain isolated from hemorrhagic colitis, Ottawa, ON, Canada), O157:H7 ATCC 35150 (EcO2; Shiga toxin-producing strain isolated from hemorrhagic colitis, Oregon and Michigan, USA), and the non-pathogenic strain E. coli ATCC 8739 (Ec) were used. All strains were cultured overnight in BHI broth (Difco, BD Diagnostics, Franklin Lakes, NJ, USA) at 37 °C. Pseudomonas fluorescens ATCC 13525 (Pf; isolated from pre-filter tanks and town water works, Reading, UK) was cultured in BHI at 25 °C for 24 h.

Bacterial cultures were grown at 37 °C until a population of 10⁶ CFU/mL was reached as previously described [38]. Briefly, serial dilutions were prepared in 0.1% sterile peptone water (Difco) and 1 ml aliquots from adequate dilutions were added to 250 ml blue capped bottles containing 100 ml of 10% reconstituted sterile skimmed milk until populations of ca. 10³ CFU/mL were achieved. These populations were evaluated by spreading onto TSA (Difco, BD Diagnostics, Franklin Lakes, NJ, USA) plates and incubating at 37 °C for 48 h.

2.2. Co-Cultures and Enumeration

A first 4-level factor (EcO1, EcO2, Ec, and Pf strains) and a second 4-level factor (7, 13, 19 or 25 °C) were used for a 4 × 4 full factorial experiment. Starting concentrations of ca. 10⁴ CFU/mL were selected and co-cultures of EcO1, EcO2, Ec, and Pf were prepared and stored at 7, 13, 19 or 25 °C. Actual co-culture starting populations of 3.9–4.1, 4.0–4.1, 4.0–4.1, and 3.9–4.1 log CFU/mL were obtained for EcO1, EcO2, Ec, and Pf, respectively. Single cultures of EcO1, EcO2, Ec, and Pf were cultivated with the same initial populations. Control cultures of un-inoculated skimmed milk were prepared and stored under the same conditions. Assays were carried out in triplicate.

Cultures were at 0, 2, 4, 6, 12, or 24 h, and 2, 4, 8, 12, 16, 20, 28 days. Aliquots (0.1 mL) were surface-plated onto MacConkey Sorbitol Agar (Difco, BD Diagnostics, Franklin Lakes, NJ, USA) and Fluorocult VRB-Agar (Merk, Darmstadt, Germany) or onto Pseudomonas Agar F or Flo Agar (Difco, BD Diagnostics, Franklin Lakes, NJ, USA). For Escherichia spp. counting, plates were incubated at 37 °C for 18–24 h, and random colonies were serologically confirmed using the E. coli O157 Latex Test Kit (Oxoid, Thermo Fisher Scientific, Basingstoke, UK). P. fluorescens colonies were counted after an incubation at 35 °C for 24–48 h.
2.3. Bayesian Modeling of Microbial Interactions

Plate counts of *E. coli* and *P. fluorescens* were transformed to decimal logarithmic values. The lag time (\(\lambda\)), the maximum population density (\(N_{\text{max}}\)), and the time to reach (\(t_{\text{tr}}\)) populations of 6 or 8 log CFU/mL were estimated for each culture using the DMFit, ComBase [39]. Then, a modified generic primary growth model [40] was selected:

\[
\frac{dN_t}{dt} = \frac{d\left(\ln(N_t)\right)}{dt} = \mu_{\max} \alpha_t f_t
\]

where \(\frac{dN_t}{dt}/N_t\) is the relative or instantaneous growth rate of the microorganism, \(N_t\) is the cell concentration in a bacterial culture at time \(t\), and \(\mu_{\max}\) is the maximum growth rate. The term \(\alpha_t\) is an adjustment function, and \(f_t\) is a logistic inhibition function for two-species mixed cultures [41]:

\[
\alpha_t = \begin{cases} 
0, & t < \lambda \\
1, & t \geq \lambda 
\end{cases}
\]

\[
f_t = 1 - \frac{Na_t + Nb_t}{N_{\text{max}}}
\]

where \(\lambda\) is the lag time, \(Na\) and \(Nb\) are the cell concentration of the microorganisms a or b in co-culture at time \(t\), and \(N_{\text{max}}\) is the total carrying capacity (both species). For EcO1 cultures the model can be re-defined:

\[
\frac{dEcO1}{dt} = \frac{d\left(\ln(EcO1_t)\right)}{dt} = \mu_{EcO1} \alpha_t \left(1 - \frac{EcO1_t}{EcO1_{\text{max}}}\right)
\]

\[
\frac{dEcO1}{dt} = \frac{d\left(\ln(EcO1_t)\right)}{dt} = \mu_{EcO1(Pf)} \alpha_t \left(1 - \frac{EcO1_t + Pf_t}{N_{\text{max}}}\right)
\]

where \(\mu_{EcO1}\) (3a) and \(\mu_{EcO1(Pf)}\) (3b) are the maximal growth rates of EcO1 cultured alone or in the presence of *P. fluorescens*, respectively. Similar approaches to the equations (3a–b) were done for the cultures of EcO2 (\(\mu_{EcO2}\), \(\mu_{EcO2(Pf)}\)), Ec (\(\mu_{Ec}\), \(\mu_{Ec(Pf)}\)), and *P. fluorescens* (\(\mu_{Pf}\), \(\mu_{Pf(EcO1)}\), \(\mu_{Pf(EcO2)}\), and \(\mu_{Pf(Pf)}\)).

When the cultures reached their maximal values, a decline period was observed. The decline phase was modeled alone with a modification of equations (3a–b), i.e., \(\mu\) was replaced by the negative-sign parameter \(k\) in order to reflect the negative slope of that survival growth section.

The approach above assumes deterministic behavior, but an error term may be introduced to reflect the influence of factors outside the experimental design. Thus, the observed concentration of bacteria at time \(t\) may modelled as \(N_t^* = N_t + \epsilon_t\), where \(N_t\) is the population of EcO1, EcO2, Ec, or Pf cultured alone or in co-culture, and \(\epsilon_t\) is a normally distributed error term with zero mean and constant variance equal to \(\sigma_t^2\): \(N_t^* \sim \text{Normal}(N_t, \sigma_t)\).

A Bayesian estimation of the parameters for computing the posterior distribution of parameters of the model was carried out. The estimated parameters are shown in Figure 1 as circles: The growth rates of the microorganisms cultured alone (\(\mu_{EcO1, Ec, Pf}\)), the 2-species mixtures (\(\mu_{EcO1(EcO2), EcO2}, \mu_{EcO1(Pf), Pf}, \mu_{Ec(Pf), Pf}\)), and the standard deviation of errors \(\sigma_t\); the other terms are constants and are shown as squares. Decline rates and the 95% credible intervals were also estimated based on the posterior distribution of parameters from equations 3a–b. The estimation of parameters, by means of a Bayesian methodology, is undertaken by simulating the posterior distribution of the model parameters, which includes the likelihood of the experimental data (assuming lognormality) and the prior distributions of the parameters. A general introduction to this methodology in differential equations for biological systems can be seen in [42].

The Runge-Kutta method was used to discretize the system of differential equations [43]; then the system was included in a probabilistic model and the Hamiltonian Monte Carlo method (HMCM) was used for parameters estimation [44] generating samples from the posterior distributions of parameters \(\mu_t\) and \(\sigma_t\) [45]. In each iteration of the HMCM sequence it is calculated a discretized version of equations (3a) and (3b) by means of the Runge-Kutta procedure for determining the likelihood of the experimental data. After the convolution with the prior distributions of parameters
it is obtained a sequence of values of the posterior distribution. Then, the means and intervals based on these draws are obtained and shown as the final estimates of the parameters. R [46] via Rstan [47] was used for algorithmic programming. Codes are available from author JMM.

![Figure 1](image-url)

**Figure 1.** Bayesian model directed acyclic graph (DAG). Circles: random variables. Squares: constants (initial parameters of the distributions of the variables). Arrows: conditional dependence. Obs. j-1: observed data of *E. coli* spp. (EcO1, EcO2, and Ec), and *P. fluorescens* (Pf). *µ*: microorganisms’ growth rates with Normal distribution (mean *m* and standard deviation *s*). *σ*: standard deviation of errors with a Gamma distribution (parameters *α*).

3. Results

3.1. Bayesian Modelling of Microbial Interactions

Bayesian inference examples of growth and decline periods for *E. coli* O157:H7 LCDC 86-51 (EcO1), *E. coli* O157:H7 ATCC 35,150 (EcO2), and non-pathogenic *E. coli* (Ec) co-cultured with *P. fluorescens* (Pf) at 7 or 25 °C in skimmed milk are shown in Figure 2, Figure S1, and Figure S2, and in Figure 3, Figure S3, and Figure S4, respectively. As a comparison, growth and decline periods from EcO1 cultured alone are also shown (Figure 2; Figure 3). Figure 4 shows the *µ* estimates from *E. coli* spp. co-cultured with *P. fluorescens* or single-cultured at 7, 13, 19, or 25 °C. The highest *µ* values were detected at 19 and 25 °C. It is interesting to observe the differences on the growth rates between *E. coli* spp. and *P. fluorescens* cultured alone: a psychrotrophic bacteria such as *P. fluorescens* did not show the lowest growth rates at any temperature except for 25 °C; however, *E. coli* spp. showed it at 7 and 13 °C. Co-cultured *P. fluorescens* showed similar growth rates as when cultured alone. The effect of *P. fluorescens* on the growth rate of *E. coli* strains appears to be greater at low temperatures (7 and 13 °C) increasing *E. coli* spp. *µ* values. At higher temperatures (19 and 25 °C) *P. fluorescens* does not seem to cause the same effect. Table S1 shows posterior means of the parameters and the limits of the credible intervals of growth rates (*µ*EcO1, *µ*EcO2, *µ*Ec, or *µ*Pf) and decline rates (*k*EcO1, *k*EcO2, *k*Ec, or *k*Pf) of *E. coli* spp. and *P. fluorescens* cultured alone or in co-cultures. The *E. coli* spp. co-cultures show the lowest *µ* values at 7 and 13 °C ranging from 0.563 day⁻¹ for the EcO1(Pf) co-cultures at 7 °C to 0.945 day⁻¹ for the Ec(Pf) co-cultures at 13 °C. At 19 and 25 °C the *µ* values were similar ranging from 1.358 day⁻¹ for the EcO1(Pf) co-cultures to 2.178 day⁻¹ for the Ec(Pf) co-cultures at 25 °C.
Figure 2. Bayesian inference of growth periods of co-cultures of *E. coli* spp. with *P. fluorescens* at 7 or 25 °C in skimmed milk. The 95% Highest Posterior Density intervals (2.5 and 97.5%) are shown. Points are the original data: mean and standard deviation are shown. Growth periods from EcO1 cultured alone are also shown.
Figure 3. Bayesian inference of decline periods of co-cultures of *E. coli* spp. with *P. fluorescens* at 7 or 25 °C in skimmed milk. The 95% Highest Posterior Density intervals (2.5 and 97.5%) are shown. Points are the original data; mean and standard deviation are shown. Decline periods from EcO1 cultured alone are also shown.
Figure 4. Scatter plots of the growth rates (μ mean) from *E. coli* spp. co-cultured with *P. fluorescens* (EcO1(Pf), EcO2(Pf), and Ec(Pf)) or single-cultured (EcO1, EcO2, and Ec) at 7, 13, 19 or 25 °C. Growth rates from *P. fluorescens* co-cultured (Pf(EcO1), Pf(EcO2), and Pf(Ec)) or single-cultured (Pf) are also shown.

At 7 °C, decreasing populations of the three *E. coli* strains cultured alone were not detected (Table S1). Decreasing populations (k values) were not found for EcO2 and Ec strains single-cultured, and for EcO1(Pf), EcO2(Pf) and Ec(Pf) co-cultures at 13 °C. The positive k values are included within the
Highest Posterior Density (HPD) intervals between a negative 2.5% interval value and a positive 97.5% interval value. The zero value is in the interval meaning that the estimated $k$ values are not significantly different from zero, i.e., there is not growth nor decline with a 97.5% of confidence, and the populations are stable. The fastest $k$ decline rates were observed in the EcO1(Pf) and Ec(Pf) co-cultures at 19 °C (−1.804 day$^{-1}$ and −1.709 day$^{-1}$, respectively). The higher $k$ decline rates from single-cultured strains were found in EcO2 cultures at 19 °C (−0.233 day$^{-1}$) and 25 °C (−0.247 day$^{-1}$).

The standard deviations from the survival curves, i.e., with growth and decline periods included, are shown in Figure 5, and Table S2. Table S2 shows posterior means of the parameters and the limits of the credible intervals (2.5% and 97.5%) of the standard deviations for growth rates ($\sigma_{\text{co}}$, $\sigma_{\text{EcO2}}$, $\sigma_{\text{Ec}}$, or $\sigma_{\text{EcO1}}$) and decline rates ($–\sigma_{\text{EcO1}}$, $–\sigma_{\text{EcO2}}$, $–\sigma_{\text{Ec}}$, or $–\sigma_{\text{EcO1}}$) of E. coli spp. and P. fluorescens cultured alone or in co-cultures. Standard deviation is read as the predicted concentrations’ random error of the microorganisms’ real observations.

![Figure 5](image-url) Scatter plot of the standard deviations for growth ($\sigma$) from E. coli spp. co-cultured with P. fluorescens (EcO1(Pf), EcO2(Pf), and Ec(Pf)) or single-cultured (EcO1, EcO2, and Ec) at 7, 13, 19 or 25 °C.

3.2. Estimation of the N$_{\text{max}}$ and the ttr

The maximum population density ($N_{\text{max}}$) of E. coli spp. and P. fluorescens in single cultures or co-cultured in milk are shown in Table S3. The lowest E. coli spp. $N_{\text{max}}$ values were observed at 7 °C (4.4–4.8 log CFU/mL in single cultures and 5.2–5.4 log CFU/mL in co-cultures); at 13, 19, or 25 °C, the $N_{\text{max}}$ values were similar for all co-cultures (8.0–8.5 log CFU/mL in single cultures and 7.9–8.5 log CFU/mL in co-cultures). The $P. fluorescens$ $N_{\text{max}}$ values were similar for all co-cultures at all temperatures (8.9 log CFU/mL).

The time to reach (ttr) a population of 6 or 8 log CFU/mL is shown in Figure 6 and Table S3. These populations were found just before the carrying capacities were reached, and both fall within the linear period of the exponential growth where rates show a Log-Normal distribution regardless of the environmental conditions and the initial population of microorganisms [48–50]. At 7 °C E. coli spp. did not reach 6 log CFU/mL whether single-cultured or co-cultured. At 13 °C all E. coli spp. cultures reached 6 log CFU/mL at 1.60–2.24 day; similar results were found at 19 or 25 °C with lower ttr 6 log values showing a faster growth: 0.56–0.64 day or 0.32–0.40 day, respectively. All $P. fluorescens$ cultures reached ca. 6 log CFU/mL after 0.56–0.72 day at 7 °C; the ttr 6 log results at 13 °C were slightly higher (0.64–1.12 day), decreasing at 19 and 25 °C and showing faster growth: 0.56–0.64 day and 0.40–0.48 day, respectively. The ttr 6 log values of $P. fluorescens$ were lower than those from E. coli strains at all temperatures, indicating faster growth. All E. coli spp. and $P. fluorescens$ cultures were able to reach a population of 8 log CFU/mL, except E. coli spp. at 7 °C. At 13 °C all E. coli spp. cultures reached
8 log CFU/mL at 3.36–3.68 d; lower results were found at 19 or 25 °C indicating a faster growth: 1.04–1.12 day or 0.56–0.96 day, respectively. All *P. fluorescens* cultures reached at 8 log CFU/mL after 0.96–1.04 d at 7 °C; the ttr 8 log results at 13, 19, or 25 °C were lower showing a faster growth: 1.44–1.76 day, 0.96–1.04 day, or 0.56–0.88 day, respectively. The *P. fluorescens*’s ttr 8 log values were lower than those from *E. coli* strains at all temperatures, indicating overall faster growth.

**Figure 6.** Time to reach (ttr; d) a population density of 6 or 8 log CFU/mL from *E. coli* spp. co-cultured with *P. fluorescens* (EcO1(Pf), EcO2(Pf), and Ec(Pf)) or single-cultured (EcO1, EcO2, and Ec) at 7, 13, 19 or 25 °C. Standard Errors are shown.

### 4. Discussion

The presence of *E. coli* O157:H7 strains in refrigerated food such as milk depicts a health risk for the consumers. The native microbiota or the presence of protective cultures could compete with the pathogens and help in controlling *E. coli* O157:H7 strains during the processing and storage of refrigerated food [31,51]. *Pseudomonas* spp. could be important competitors in perishable refrigerated food products due to their psychrotrophic profile (able to grow at 0–15 °C) [31,52].

Antagonistic microorganisms (e.g., *Pseudomonas* spp.), may be useful in the control of *E. coli* O157:H7 growth. According to Samelis and Sofos [31], *E. coli* O157:H7 co-cultured with *Pseudomonas* sp. grew faster as the temperature increased from 10 to 15 or to 25 °C in TSB broth. These authors found that the pathogen inhibition was enhanced in co-cultures grown at 10 to 15 °C with 1% of added glucose. At 25 °C the inhibition was enhanced even without added glucose. Previously, Janisiewicz et al. [53] reported that *P. syringae* inoculated into apple injuries inhibited the growth of *E. coli* O157:H7. Similar results were found when a high level of ground beef native flora inhibited the growth of *E. coli* O157:H7 at 10–12 °C [54,55]. Samelis and Sofos [31] found that the maximum population density of *E. coli* O157:H7 was suppressed in co-culture with *Pseudomonas* at 10, 15, and 25 °C. These results are in agreement with the Jameson Effect [55]; indeed, the inhibition of a population not in its stationary phase by another population in it is observed [30,55], i.e., the competition in food mixed populations is restricted to the limitation of the maximum population, with no effect on the growth rate. Our work supports the Jameson-effect hypothesis as the growth rates of *E. coli* species seems not to be affected by *P. fluorescens*. Similar results were found by Buchanan and Bagi [18] when *P. fluorescens* suppressed *Listeria monocytogenes* growth by inhibiting its maximum population density at low incubation temperatures (4 °C); the inhibition was less
evident at higher temperatures (12 and 19 °C). McKellar [56] also reported that a raw milk isolate of P. fluorescens suppressed the growth of E. coli O157:H7 in nutrient broth at 22 °C only when P. fluorescens had reached its maximum population. Similar results were found in a previous study in co-cultures of Listeria spp. with P. fluorescens [38] but without differences between low and high temperatures, as Buchanan and Bagi [18] did. Samelis and Sofos [31] reported that E. coli O157:H7 co-cultured with Pseudomonas reached a population of ca. 6 log CFU/mL after −2.6 d at 10 °C, not achieving a population of 8 log CFU/mL along the study (14 day); the same co-culture reached a maximum of 7 log CFU/mL (~6.1 day) when the TSB broth was supplemented with 1% of glucose. In our study E. coli spp. did not reach 6 or 8 log CFU/mL at 7 °C. Samelis and Sofos [31] found that E. coli O157:H7 co-cultured at 15 °C with Pseudomonas reached populations of 6 or 8 log CFU/mL after −0.5 or 2 d, respectively and when the co-cultures were supplemented with 1% of glucose E. coli O157:H7 achieved populations of 6 or 8 log CFU/mL after 1.2 or 7 d, respectively. We found slightly slower growth in our study at 13 °C with a trr 6 log of 1.6–2.2 d, and a trr 8 log of 3.5–3.7 d. These authors [31] did not detect changes in pH along the incubation period (14 day) irrespective of the temperature and the type of culture: pH values of 7.3–7.4; in contrast, pH reductions were pronounced when 1% of glucose was added to the medium decreasing to values of 5.0–6.0. The pH values in single cultures or in co-cultures in our study decreased along the study from 6.7–6.8 to about 6.5 after 28 day at 7 or 13 °C (data not shown). At 19 or 25 °C the pH decreased until values of about 4.0–4.5 (data not shown) at the end of the study probably due to E. coli use of the lactose from the milk.

Lebert et al. [57] found that the growth of L. monocytogenes and L. innocua were not affected by Pseudomonas spp. at 6 °C on decontaminated meat but Pseudomonas spp. did affect L. innocua on native-microbiota contaminated meat – when Pseudomonas achieved their stationary phase Listeria was able to grow. These results are in contrast with previous [38,58,59] and current results which found that P. fluorescens exerts similar inhibitory effects on the E. coli strains studied. Besse et al. [58] noted interactions at the end of the exponential phase—when a strain reached its carrying capacity the growth of both strains stopped. McKellar [56] reported that nutrient limitation was the cause of the competition between Pseudomonas and E. coli O157:H7. But quorum sensing stimuli has also been suggested as a mechanism [16,60–63]. Once a faster growing microorganism reaches its maximum population, the production of signaling molecules also reaches its maximum, indicating to other species of the mixed culture that the carrying capacity of the culture has been achieved. Chu et al. [60] also showed how E. coli indole production inhibited P. aeruginosa factors important for competition.

As P. fluorescens constitutes a major component of native bacteria associated with fresh and minimally processed produce, Liao [32] studied the control of foodborne pathogens by P. fluorescens AG3A and Bacillus YDI both isolated from fresh peeled baby carrots. Both strains reduced the growth of L. monocytogenes, Yersinia enterocolitica, Salmonella enterica, and E. coli O157:H7 at 20 °C but not at 10 °C. Olaya et al. [34] reported a moderate inhibition of E. coli O157:H7 by P. fluorescens on spinach leaf surfaces. These strains showed similar behaviors when they were co-cultured with nutrient restrictions at 10–35 °C for 48 h [35]; these authors found an E. coli O157:H7 trr 6 log of 1.5 d at 20 °C, without reaching a population of 8 log CFU/mL; at 35 °C the trr 6 log was about 1.1 d, and the trr 8 log 1.6 d.

In our experiments P. fluorescens grew faster than E. coli spp. at 7 and 13 °C cultured alone as well as in co-culture, with higher μmax values and lower trr 6 or 8 log; however, P. fluorescens did not affect the μmax1, μmax2, and μmax3 values. Similar behavior was observed when L. monocytogenes were co-cultured with Lactobacillus sakei [64] or P. fluorescens [38] together with higher μmax of both competitors; in contrast the current study did not find higher μmax of P. fluorescens single cultured or co-cultured with E. coli spp. At 19 and 25 °C the trr 6 or 8 log of P. fluorescens were lower than those of E. coli spp. and the μmax values were similar between single cultures and co-cultures showing also similar μmax; slight maximum population increases (<1 log CFU/mL) of P. fluorescens at 25 °C were observed. These results are not consistent with the Jameson Effect [30,55,65] with regard to the inhibition of one species by another that has reached the stationary phase. There is no correlation.
between the μ and its effect on the maximal population densities of E. coli spp. (Pearson’s coefficient correlation of −0.407). The values of E. coli spp. N\text{max} were high at all temperatures except at 7 °C (N\text{max} of about 5.2–5.4 log CFU/mL), so the increasing μ values did not increase E. coli spp. N\text{max} together with the increase of the temperatures. It would be possible to consider the fermentation of milk lactose by the E. coli spp. as a “high risk, high reward” strategy in the two-species communities studied [66,67]. E. coli spp. must engage additional competitive mechanisms to remain viable such as lactose fermentation [68] although it was far from the aim of this work to explore it. These interactions could also be related to physical location or resource usage overlapping between both populations [67]. Another possible explanation for the absence of the Jameson effect at the higher temperatures studied could be a “counterattack strategy”. Some authors have reported that P. aeruginosa suffering the attack from Vibrio cholerae or Acinetobacter baumannii’s type VI secretion system (T6SS) respond striking back with its own T6SS [69]. The T6SS is a multiprotein contractile-weapon complex that participates in interbacterial competition delivering toxins into both prokaryotic and eukaryotic cells. The T6SS complex does occur in Escherichia coli and Salmonella [70] including enterohemorrhagic E. coli O157:H7 [71]. Decoin et al. [72] described a T6SS involved in P. fluorescens bacterial competition against the potato tuber pathogen Pectobacterium atrosepticum. Although the objective of our study is far from the description of a T6SS P. fluorescens activity against E. coli spp., the results provide evidence for a bacterial “tit-for-tat” [73] or “T6SS dueling” [74] evolutionary strategies that control interactions among different bacterial species.

5. Conclusions

The aim of this work was to study and model the dynamics of the competition between Escherichia coli O157:H7 and Pseudomonas fluorescens co-cultured at 7, 13, 19, and 25 °C in milk. A parametric Bayesian approach was used assuming that the parameters μ (growth rate), k (decline rate), σ (standard deviation of the growth rates), and −σ (standard deviation of the decline rates) are random variables with their own prior distributions. Model results and confidence intervals are based on a probabilistic background. The highest E. coli O157:H7 populations were similar at all temperatures, except at 7 °C: E. coli spp. strains reached their maximal population of 4 log CFU/mL cultured alone, and 5 log CFU/mL co-cultured with P. fluorescens. At 13, 19, and 25 °C E. coli spp. reached their maximal population of 8 log CFU/mL single cultured and co-cultured, with times to reach a population of 6 log CFU/mL after ~48 h at 13 °C or ~24 h at 19 and 25 °C. P. fluorescens achieved its maximal densities of 8–9 log CFU/mL in all cultures at all temperatures, with similar times to reach a population of 6 or 8 log CFU/mL. The results obtained show that the growth rate of P. fluorescens has no direct correlation with its effect on the maximal population of E. coli strains. Modeling the behavior of bacterial communities helps in understanding their dynamics. The inhibition of foodborne pathogens with the use of some species from the natural food microbiota as probiotics may be a tool to improve the safety of refrigerated foods such as milk and dairy products.

Supplementary Materials: The following are available online at www.mdpi.com/2304-8158/9/3/331/s1, Table S1: Bayesian estimates of the posterior means and Highest Posterior Density intervals (HPD: 2.5 and 97.5%) of the growth (μ; d⁻¹) and decline (k; d⁻¹) rates of E. coli O157:H7 LCDC 86-51, E. coli O157:H7 ATCC 35150, non-pathogenic E. coli, and P. fluorescens cultured alone (EcO1, EcO2, Ec, Pf), or co-cultured (EcO1 + Pf, EcO2 + Pf, Ec + Pf) at 7, 13, 19 or 25°C. Table S2: Bayesian estimates of the posterior means and Highest Posterior Density intervals (HPD: 2.5 and 97.5%) of the standard deviations for growth (σ) and decline (−σ) periods of E. coli O157:H7 LCDC 86-51, E. coli O157:H7 ATCC 35150, non-pathogenic E. coli, and P. fluorescens cultured alone (EcO1, EcO2, Ec, Pf), or co-cultured (EcO1 + Pf, EcO2 + Pf, Ec + Pf) at 7, 13, 19 or 25 °C. Table S3: Maximal population density (N\text{max}; log CFU/mL) and time to reach (ttr; d) a population density of 6 or 8 log CFU/mL of E. coli spp. strains and P. fluorescens cultured alone or in co-culture at 7, 13, 19, or 25 °C. Figure S1: Hamiltonian Monte Carlo Method (HMC) diagnosis plots of the growth rates of E. coli O157:H7 LCDC 86-51 (EcO1), E. coli O157:H7 ATCC 35150 (EcO2) or E. coli ATCC 8739 (Ec) co-cultured with P. fluorescens (EcO1(Pf), EcO2(Pf), or Ec(Pf)) at 7 °C. Panels show three plots for the growth rate parameter: Traces or value estimated in each step of the HMM (left); the parameter posterior distributions (middle); and the autocorrelation functions for the parameter estimates (right). μ[1] and μ[2] are the growth rates of E. coli spp or P. fluorescens, respectively. Figure S2: Hamiltonian Monte Carlo Method (HMC) diagnosis plots of the decline rates of E. coli O157:H7
LCDC 86-51 (EcO1), *E. coli* O157:H7 ATCC 35150 (EcO2) or *E. coli* ATCC 8739 (Ec) co-cultured with *P. fluorescens* at 7°C. See legends and explanations in Figure S1. k[1] and k[2] are the decline rates of *E. coli* spp or *P. fluorescens*, respectively. **Figure S3:** Hamiltonian Monte Carlo Method (HMC) diagnosis plots of the growth rates of *E. coli* O157:H7 LCDC 86-51 (EcO1), *E. coli* O157:H7 ATCC 35150 (EcO2) or *E. coli* ATCC 8739 (Ec) co-cultured with *P. fluorescens* at 25°C. See legends and explanations in Figure S1. **Figure S4:** Hamiltonian Monte Carlo Method (HMC) diagnosis plots of the decline rates of *E. coli* O157:H7 LCDC 86-51 (EcO1), *E. coli* O157:H7 ATCC 35150 (EcO2) or *E. coli* ATCC 8739 (Ec) co-cultured with *P. fluorescens* at 25°C. See legends and explanations in Figures S1 and S2.

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