



ORIGINAL ARTICLE

Modelling of the growth of populations of *Listeria monocytogenes* and a bacteriocin-producing strain of *Lactobacillus* in pure and mixed cultures

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The cell growth biokinetics of Listeria monocytogenes and a bacteriocin-producing strain of Lactobacillus sake were studied. The organisms were grown both separately and together in a broth system. It was observed that Lact. sake was better suited to growth at low pH than L. monocytogenes. The presence of bacteriocin impacted on L. monocytogenes, by quickly reducing the number of these microbes in mixed populations. A model for the growth of L. monocytogenes and Lact. sake populations grown separately and in mixed culture is proposed. The model includes a novel approach for better describing the effect of pH change, the ability of Lact. sake to produce bacteriocin and the effect of bacteriocin on L. monocytogenes in the broth culture. Additionally, terms are introduced that describe the competitive effect of one population on the other. The model is fitted to data collected for the growth of each population, either grown alone or together. There were no significant differences between the parameter estimates for each replicate, supporting the hypothesis that the proposed model is a useful representation of the growth and interaction of the two microbial populations.

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Introduction

An understanding of the interactions between the different micro-organisms present in food is important for food safety. The mechanisms by which such micro-organisms can interact have been considered in the literature for a long time (Fredrickson 1977), and include com-

petition for nutrients, tolerance of waste products and the production of inhibitory substances effective against other micro-organisms. Interactions between different populations may have many consequences, but of particular interest to food microbiology is the feature that a population of pathogenic bacteria can be suppressed through competition with a benign species of bacteria, and that this competition can be encouraged to increase food safety. Mathematical modelling represents one way of understanding these interactions

Received: ??????

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and, to this end, general models of interacting microbes in foods have been most recently considered by Dens et al. (1999) and Vereecken et al. (2000).

One of the primary mechanisms by which pathogens may be suppressed by competing microflora is through the production of bacteriocins by non-pathogenic lactic acid bacteria and other bacteria. Bacteriocins are diffusible, proteinaceous compounds active against closely related bacteria. Bacteriocins, and particularly those produced by the lactic acid bacteria, have received considerable interest in the food industry because they are naturally occurring compounds and some, such as nisin, have 'generally recognized as safe' (GRAS) status in many countries (Gould 1996, Muriana 1996). Furthermore, inoculation of bacteriocin-producing lactic acid bacteria into foods to control the growth of pathogens such as *Listeria monocytogenes* has been successfully demonstrated by Stecchini et al. (1992) in ham, Winkowski and Montville (1992) in meat gravy, Schillinger et al. (1991) in meat, and Nilsson et al. (1999) in smoked salmon.

To understand the interaction of pathogenic and bacteriocin-producing lactic acid bacteria further, this paper proposes and tests a mathematical model for the interaction between a bacteriocin-producing *Lactobacillus sake* strain and *L. monocytogenes* in liquid culture.

Materials and Methods

Growth conditions and sampling

A flask containing 250 ml of sterile de Man, Rogosa and Sharp (MRS, Difco, Detroit, Michigan, USA) broth (adjusted to pH 7.0 using NaOH), preheated to 20°C, was inoculated with approximately 100 cfu ml⁻¹ *L. monocytogenes* L70 that had been grown for 18 h at 24°C in 10% CO₂ in MRS broth. A second flask was inoculated in the same fashion with *Lact. sake* 706 (Schillinger and Lücke 1989), which produces the bacteriocin sakacin A which is active against *L. monocytogenes*. A third flask was inoculated in the same fashion with both organ-

isms. The three flasks were incubated at 20°C in a shaking water bath. Appropriately sized samples were taken at intervals ranging between 2 and 28 h and assayed for pH, bacterial numbers and bacteriocin level. The experiment was performed in duplicate.

Microbiological analysis

At each sampling interval, 1 ml of culture was removed and assayed for pH using a PHM210 Standard pH meter (Radiometer, Copenhagen, Denmark). Tenfold dilutions were performed on samples of 100 µl from the *L. monocytogenes*—only flask and the flask containing both organisms. Diluted samples were plated on *Listeria*-selective Modified Oxford (MOX) agar (Difco, Detroit, Michigan, USA). Similarly, samples from the *Lact. sake*—only flask and the flask containing both organisms were plated on to MRS agar which was selective for lactic acid bacteria by adjusting to pH 5.7 with HCl. Plates were incubated for 24 h (MOX) or 48 h (MRS) at 24°C in 10% CO₂ and colonies counted.

Bacteriocin activity in the culture supernatant at each sampling time was determined using a well diffusion assay described by Galvin et al. (1999). Briefly, a total of 500 µl of the culture was centrifuged at 10 000 g for 20 min and the culture supernatant adjusted to pH 7.0 with 6N NaOH. The supernatant was then subjected to a serial two fold dilution series in sterile MRS. Molten agar (20-ml aliquots) at 48°C was inoculated with 50 µl of an overnight culture of *L. monocytogenes*, poured into a sterile Petri dish and allowed to solidify. Wells were cut into the agar using a sterile corkborer 4.6 mm in diameter, and 50 µl of each dilution of the supernatant was dispensed into the wells. Plates were incubated at 30°C overnight and bacteriocin activity calculated as the inverse of the last dilution showing a clear zone of inhibition of the *L. monocytogenes*. Activity units were expressed per millilitre.

The model

To understand the interaction effect among bacterial populations better it is convenient to

model independent growth for each population and compare this with the growth of each population in mixed culture. Cell growth of both *L. monocytogenes* and *Lact. sake* are described by the commonly used Logistic equation (Lejeune et al. 1998, Peleg 1997). Factors such as nutrient availability, temperature and pH affect the growth rate and maximum population size. For these experiments all external factors are modelled as constants. Hence, the model includes only those factors modified internally, which in this study is the pH of the growth medium. In the system under consideration the change in pH is caused by metabolic activity of the micro-organisms. The growth of the populations is affected by the pH of the medium. In particular, as the pH deviates from optimum the maximum population size decreases as the square of the deviation. Evidence for this relationship between microbial population growth and pH has been presented by Cole et al. (1990). The change in pH depends on size n_L of the population and its instant state, characterized by dn_L/dt . In the simplest version the only characteristic of the microbial state (lag, exponential or steady state) is the current growth rate. The mathematical model for population size of *L. monocytogenes* (n_{Lis}) is thus:

$$\begin{aligned} \frac{dn_{Lis}}{dt} &= g_{Lis}n_{Lis} \left(\frac{1 - n_{Lis}}{Lis_{\max}} \right) \\ \frac{dpH}{dt} &= - \left(k_{11} - \frac{dn_{Lis}}{dt} + K_{12} \frac{n_{Lis}}{Lis_{\max_0}} \right) \end{aligned} \quad (1)$$

where

$$Lis_{\max} = Lis_{\max_0} - \beta_{Lis}(pH - pH_{Lis})^2$$

For the population of *Lact. sake* the growth kinetics can be described in the same way. However, *Lact. sake* also produces sakacin A which has antimicrobial activity and which needs to be accounted for in the model. The rate of bacteriocin production by the *Lact. sake* population is maximal in the active phase of microbial growth (Hugas et al. 1995). Thus the bacteriocin production depends on both the numbers of *Lact. sake* and the current state of their growth. Taking this dependence in the simplest form, the mathematical model for the

population of *Lact. sake* (n_{Lac}) can be written in the following form:

$$\begin{aligned} \frac{dn_{Lac}}{dt} &= g_{Lac}n_{Lac} \left(1 - \frac{n_{Lac}}{Lac_{\max}} \right) \\ \frac{dpH}{dt} &= - \left(k_{21} \frac{dn_{Lac}}{dt} + k_{22} \frac{n_{Lac}}{Lac_{\max_0}} \right) \\ \frac{db}{dt} &= p_1 \frac{dn_{Lac}}{dt} + p_2 n_{Lac} - \kappa b \end{aligned} \quad (2)$$

where

$$Lac_{\max} = Lac_{\max_0} - \beta_{Lac}(pH - pH_{Lac})^2$$

The model for the growth of a mixed population builds on this construction by adding a term for the destructive effect on *L. monocytogenes* by bacteriocin and also describes how the growth rate of each population is affected by the presence of the other because of competitive interaction. Such an interaction term is a common feature of models in predictive microbiology, see, for example, the recent review by Vereecken et al. (2000). The mixed growth of *L. monocytogenes* and *Lact. sake* can then be described by the following set of equations:

$$\begin{aligned} \frac{dn_{Lis}}{dt} &= g_{Lis}n_{Lis} \left(1 - \frac{n_{Lis}}{Lis_{\max}} - \lambda_{Lis}n_{Lac} \right) \\ &\quad - vn_{Lis}b \\ \frac{dn_{Lac}}{dt} &= g_{Lac}n_{Lac} \left(1 - \frac{n_{Lac}}{Lac_{\max}} - \lambda_{Lis}n_{Lis} \right) \\ \frac{dpH}{dt} &= - \left(k_{11} \frac{dn_{Lis}}{dt} + k_{21} \frac{dn_{Lac}}{dt} + k_{12}n_{Lis} \right. \\ &\quad \left. + k_{22}n_{Lac} \right) \end{aligned} \quad (3)$$

$$\frac{db}{dt} = p_1 \frac{dn_{Lac}}{dt} + p_2 n_{Lac} - \kappa b - vn_{Lis}b$$

where

$$\begin{aligned} Lis_{\max} &= Lis_{\max_0} - \beta_{Lis}(pH - pH_{Lis})^2 \\ Lac_{\max} &= Lac_{\max_0} - \beta_{Lac}(pH - pH_{Lac})^2 \end{aligned}$$

In equations (1), (2) and (3) the parameters g_{Lis} and g_{Lac} characterize the growth rate of *L. monocytogenes* and *Lact. sake* respectively; the

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parameters λ_{Lis} and λ_{Lac} describe the influence of the population size of *Lact. sake* on the population growth of *L. monocytogenes* and vice versa. The coefficient v describes the mutual binding of the *L. monocytogenes* population and the bacteriocin. The rates of change in pH caused by microbial activity are represented by parameters k_{ij} ($i, j = 1, 2$). The parameters p_1 and p_2 are the rates of bacteriocin production, and κ is the natural degradation rate of the bacteriocin. Lis_{max_0} is the maximum population size of *L. monocytogenes* and Lac_{max_0} is the maximum population size of *Lact. sake* at the optimum pH level (pH_{Lis} and pH_{Lac}) for each organism. The empirical coefficients β_{Lis} and β_{Lac} describe changes of the Lis_{max} and Lac_{max} caused by the decrease of pH.

Statistical analysis

Equations (1), (2) and (3) cannot be solved analytically. Technically, the solution of equation set (1) consists of the solving of two simultaneous nonlinear equations in time, one equation for the population size of *L. monocytogenes*, the other for the level of pH. The solution of equation set (2) consists of three simultaneous nonlinear equations in time. One equation is for the population size of *Lact. sake*, one is for the level of pH, and the last equation is for the level of bacteriocin at any time t . Similarly, the solution of equation set (3) consists of four simultaneous nonlinear equations in time. In this case there is the addition of an equation for the population size of *L. monocytogenes* at any time to the equations for the population size of *Lact. sake* and the levels of pH and bacteriocin.

Although the equations (1), (2) and (3) cannot be solved analytically, they can be solved numerically. The estimation of parameters, based on the numerical solution, is a problem in simultaneous nonlinear regression, where parameter estimates are found by minimizing the determinant of the variance–covariance matrix of the residuals (Bates and Watts 1988). Minimizing the determinant in simultaneous regression is the analogue of minimizing the sum of squares in the case of single equation regression. The derivation is given in Bates and Watts (1988). Under normality it is also the maximum likelihood estimator.

The parameters for each replicate of microbial growth in pure cultures was found by using a genetic algorithm (Haupt and Haupt 1998) combined with successive line minimization (Press et al. 1989) to minimize the determinant of the variance–covariance matrix of the residuals.

The practice of measuring the numbers of microbes by serial tenfold dilution introduces errors of measurement because of the dilution process. This means that the measurement errors associated with high numbers of microbes are higher than for smaller numbers of microbes. Accordingly, each residual in the analysis was weighted by the number of microbes associated with it. After this the determinant of the residuals was minimized.

The model was not identified for the datasets used to obtain parameter estimates, which means that the parameter estimates would not be unique (see Seber and Wild 1989 for discussion about statistical identification in this context). Statistical identification of the equations required the introduction of some constraints on the parameters. The use of the optimum pH for growth of *L. monocytogenes* and *Lact. sake* ($pH_{Lis} = 6.8$ and $pH_{Lac} = 6.0$) as a constraint identified the equations for estimation. These optimum values for growth are typically observed and recorded in the literature (McClure et al. 1991, Franz et al. 1991).

The replicates of microbial growth in mixed culture did not have sufficient observations to estimate all the parameters in equation set (3). Thus, the parameters for the growth of each population were fixed at levels estimated for the growth in pure culture, and the extra parameters λ_{Lis} , λ_{Bac} , and v were estimated. That is, the estimates of these mixed culture parameters were conditional on the values taken by the other parameters, which were obtained from analysis of the single populations. This seems sensible because if the microbial populations do not interact, either through bacteriocin or by competition for resources, then equation set (3) reduces to the independent equation sets (1) and (2).

The measurements of bacteriocin were carried out by serial twofold dilution. This means that the figure quoted represents a lower bound on the amount present, with an upper

bound given by the next doubling in dilution. For example, a measurement of bacteriocin of 320 units represents a level of bacteriocin lying between 320 and 640 units. Thus, estimation based on sole consideration of the quoted levels of bacteriocin is prone to be low. Writing the a^{th} actual level of bacteriocin at time t as $b_a(t)$ and the quoted level of bacteriocin $b_q(t)$ gives:

$$b_a(t) = b_q(t) + x < b_{2q}(t) \quad (4)$$

where x is an unknown variable.

The estimate of the model parameters associated with bacteriocin is given by the expectation of the parameter given the value of bacteriocin, i.e. the conditional expectation $E[p_1, p_2, \kappa, \nu | b_a(t)]$. However, the value of bacteriocin is only known to be within a particular interval, and can take any value in this interval with a certain probability. Let the probability distribution for any value x within this interval be given by $P[x]$. The estimates of the parameters are then given by:

$$E \left[\int P[p_1, p_2, \kappa, \nu | b_a(t)] P[x] dx \right]$$

where E is the expectation operator, $P[x]$ is the probability distribution of x in the interval, $b_a(t)$ is given by equation (4), and the integral is taken over the range of possible values for the bacteriocin within the interval of the probability distribution P . Assume that if more than one observation of bacteriocin lies within an interval then, within this interval, these values are monotonic in time, i.e. if time $x_i < \text{time } x_{i+1}$ then $E[x_i] = E[P(x_i | x_i < x_{i+1})]$.

Assume that the levels of bacteriocin are distributed uniformly over the intervals in which they are observed, conditional on the expected values being monotonic in time within an interval. Estimates of the parameters can then be made by simulating values for bacteriocin in each interval, by sampling from a uniform distribution and calculating the estimates of the parameters for each simulation. The ensemble of simulations forms a frequency distribution for each parameter. An estimate of each parameter can be obtained from the parameter frequency distributions generated in this way.

Results

The results of the determination of the numbers of bacteria, pH of the medium and level of bacteriocin produced by *L. monocytogenes* L70 and *Lact. sake* 706 in pure and mixed culture are presented in Figs 1–3.

In pure culture (Figs 1 and 2) both species followed standard growth patterns, with a resultant marked drop in pH at or near to the end of the exponential growth phase, followed by a slow pH decrease during the subsequent stationary phase. The marked drop in pH was greater for *Lact. sake* (~ 2.5 pH units) than for *L. monocytogenes* (~ 1.8 pH units). For both strains, despite a pH decrease, numbers of cells remained constant for the 80-h duration of the study once the stationary phase had been reached. Bacteriocin levels for *L. sake* 706 increased and peaked during the late exponential phase of growth at the same time as the pH dropped, and then dropped off markedly during the subsequent stationary phase.

In mixed culture (Fig. 3), the behaviour of *L. sake* 706 was very similar to that seen in monoculture, with both pH changes and bacteriocin production following the previous trends. The behaviour of *L. monocytogenes* L70 was, however, very different to that in monoculture and, after starting growth to mid-logarithmic phase, numbers of this organism decreased rapidly until they were undetectable for the remainder of the study. This drop in numbers of *L. monocytogenes* L70 was associated with a reduction of pH and increasing levels of bacteriocin.

The estimates of the parameters and their 95% confidence limits associated with equation (1) for *L. monocytogenes* are shown in Table 1, and for equation (2) for *Lact. sake* are shown in Table 2. The 95% confidence limits overlap, showing that there are no significant differences in the parameter estimates between replications within species. The asymmetry in the confidence intervals is typical for the parameters of nonlinear models (Bates and Watts 1988).

Figure 1 shows the fit of *L. monocytogenes* numbers through time, and the change in the pH of the growth medium through time, to

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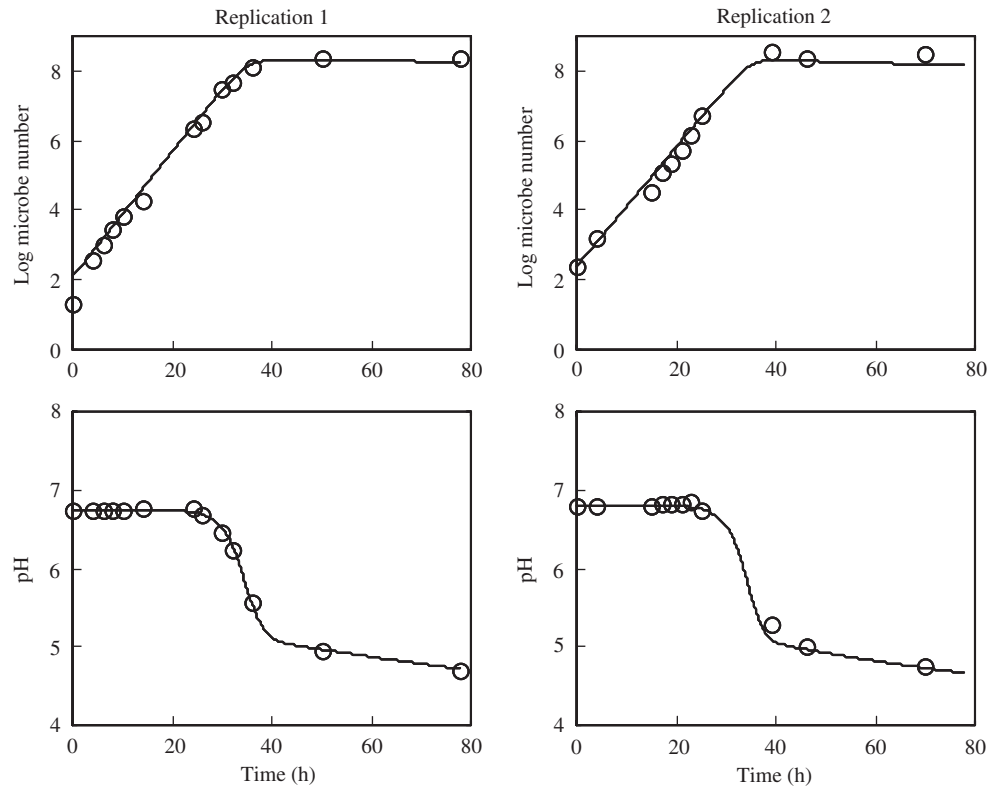


Figure 1. The estimated and measured log of the population size and environmental pH of *L. monocytogenes* through time for each of two replications. Predicted values are shown by lines and observed values by circles.

model (1) for each of the two replications. Figure 2 shows the fit to model (2) of each replication of *Lact. sake* numbers, the pH of the growth medium and the level of bacteriocin through time. Note that generally the estimates of the amount of bacteriocin are greater than the recorded amounts. This is correct since, as explained in the Materials and Methods section, the recorded amount of bacteriocin represents a lower bound on the actual amount. *L. monocytogenes* grows at a slower rate, and tends to reach a greater maximum population size than *Lact. sake*. The pH level for the *Lact. sake* growth environment falls faster than for the growth environment for *L. monocytogenes*. The estimates of the parameters associated with the generation of acid (lower pH) are an order of magnitude higher in *Lact. sake* than in *L. monocytogenes*, although overlapping 95% confidence intervals showed that this difference was not significant. The pH environ-

ment may be a factor in the maximum population size attained by each species.

There were significant ($P < 0.01$) correlations between the residuals from the number of *Lact. sake* and the residuals from the pH ($r = -0.60$). There was no significant correlation between the residuals of the population size of *Lact. sake* and the production of bacteriocin but, as already stated, the quoted values of bacteriocin are lower bounds, not measurements. Higher values of pH residuals were significantly ($P < 0.01$) associated with higher levels of bacteriocin residuals in replication 2 for *Lact. sake* grown alone ($r = 0.55$) and in replication 2 for the mixed populations ($r = 0.55$). There was a weaker insignificant correlation between pH residuals and bacteriocin residuals in replication 1 of *Lact. sake* grown alone and for replication 1 in the mixed populations.

The estimates of the interaction parameters λ_{Lis} , λ_{Lac} and ν for equations (3) for replication 1

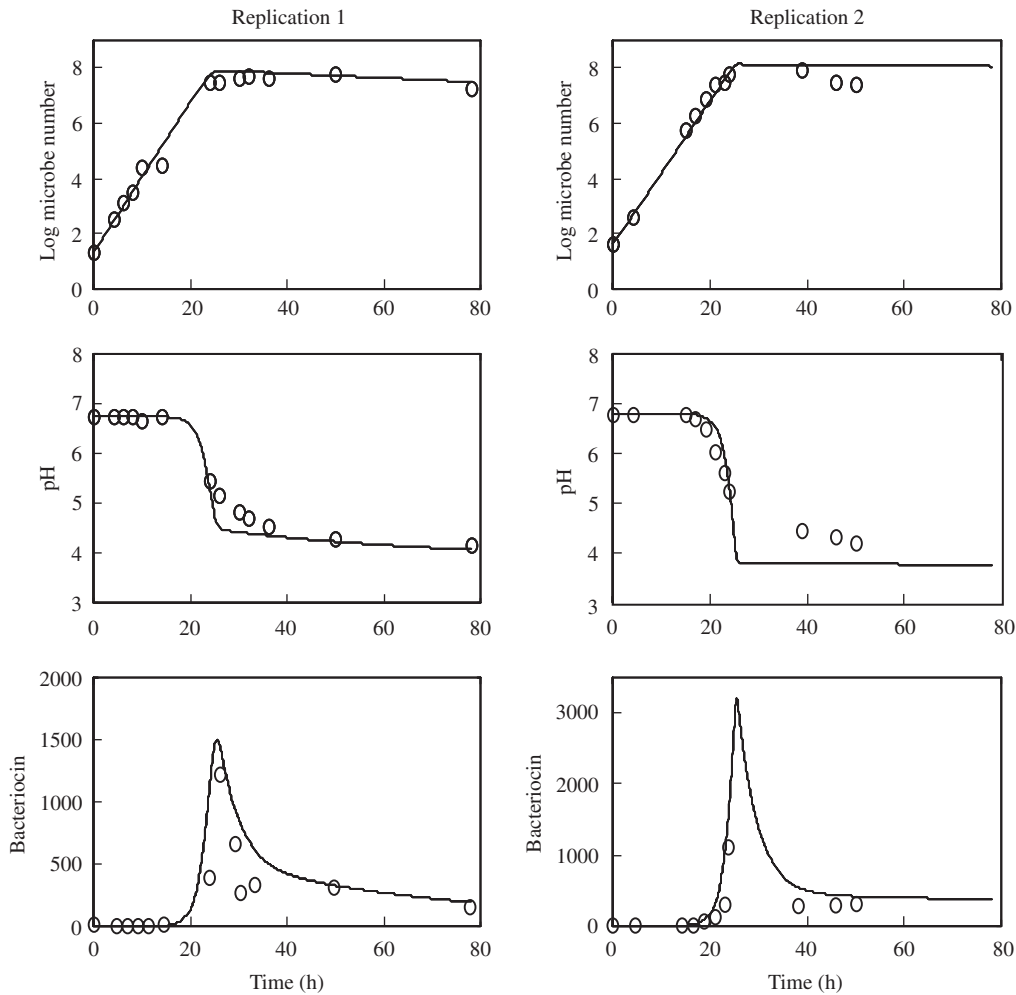


Figure 2. The estimated and measured log of the population size of *Lact. sake*, environmental pH and bacteriocin (activity units/ml) through time for each of two replications. Predicted values are shown by lines and observed values by circles.

are shown in Table 3. For all other parameters the average of the parameter estimates from the single cultures were used. Therefore the estimates of the parameters in the mixed culture are conditional on the values of the parameters for the non-interacting cultures. Figure 3 shows the fit to the microbial numbers of mixed species *Lact. sake* and *L. monocytogenes*, as well as the pH of the growth medium and the recorded level of bacteriocin for replication 1.

Discussion

The results shown in Figs 1–3 represent a typical experiment to quantify the effect of a

bacteriocin-producing lactic acid bacterium on a sensitive pathogen such as *L. monocytogenes*. The use of bacteriocin producing bacteria as starter or protective cultures in food represents a potential means to control foodborne pathogens and assure food safety (Winkowski and Montville 1992, Muriana 1996). Broth cultures of the type used in the current study represent a model system for many foods, and in particular those such as milk which permit free mixing of the components (Winkowski and Montville 1992, Breidt and Fleming 1998). Furthermore, inhibition of the bacteriocin in broth systems may be a indicator of a similar effect in other foods. To optimize the interaction of bacteriocin-producing lactic acid

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bacteria and pathogens it is necessary to understand the way they interact in relation to multiple environmental factors such as cell numbers, pH and bacteriocin production. Mathematical models represent one way in which these interactions can be better understood. The present study used a well under-

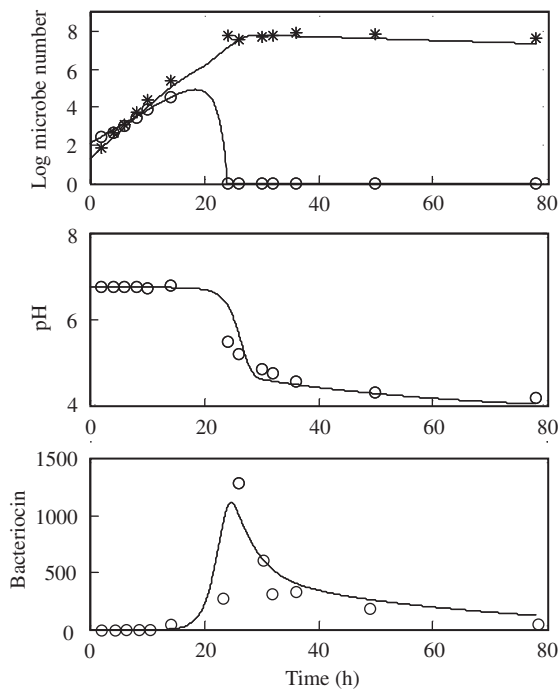


Figure 3. The estimated and measured log of the population numbers, pH and level of bacteriocin (activity units/ml) for the growth of a mixed culture of *L. monocytogenes* and *Lact. sake*. Measured values related to *L. monocytogenes* and *Lact. sake* are shown by circles and asterisks, respectively. Calculated curves are shown by lines.

stood and described bacteriocin-producing strain, *Lact. sake* 706 (Schillinger and Lücke 1989, Schillinger et al. 1991), in a mixed population with a bacteriocin-sensitive strain of *L. monocytogenes*. The interaction of these organisms was investigated using a broth modelling system to generate data, which were then modelled using a novel mathematical approach. This approach expressed the existing multiple interactions taking place in mixed microbial population growth more accurately than do current models.

The results on microbial population growth rate are similar to other studies that measured growth in both pure and mixed cultures for similar systems (Schillinger and Lücke 1989, Winkowski and Montville 1992, Muriana 1996). However, a number of features are worthy of note.

The sudden drop in numbers of *L. monocytogenes* to undetectable levels during mixed culture with the concomitant survival and growth of the *Lact. sake* strain (Fig. 3) was expected, but was more rapid and complete than for the same bacteriocin-producing strain with different *L. monocytogenes*. Since an isogenic mutant of *Lact. sake* 706 has been shown to be unable to reduce numbers of the indicator organism in broth culture despite a normal decrease in pH in a previous study (Schillinger and Lücke 1989), it seems likely that bacteriocin production is the main reason for the observed decrease in this study. In the present study, a drop in pH of the medium was observed as cell numbers increased in the pure culture (Figs 1 and 2). This pH drop was less in the case of *L. monocytogenes* (Fig. 1) than for *Lact. sake* (Fig. 2). It appears that both strains have a high resistance to acid and that the observed

Table 1. Estimates of parameters and 95% confidence limits for each replication of *Listeria monocytogenes* grown in separate culture

| Parameter | Parameter estimates (95% confidence interval) | |
|---------------|--|--|
| | Replication 1 | Replication 2 |
| g_{Lis} | 0.41 (0.39–0.42) | 0.39 (0.38–0.40) |
| Lis_{max0} | 2.200×10^8 (1.939×10^8 – 5.939×10^8) | 3.499×10^8 (2.470×10^8 – 5.470×10^8) |
| β_{Lis} | 0.011 (0.005–0.170) | 0.136 (0.055–0.215) |
| κ_{11} | 7.755×10^{-9} (5.755×10^{-9} – 1.171×10^{-8}) | 8.302×10^{-9} (6.356×10^{-9} – 1.236×10^{-8}) |
| κ_{12} | 0.009 (0.003–0.018) | 0.021 (0.013–0.0371) |

Table 2. Estimates of parameters and 95% confidence limits for each replication of *Lactobacillus sake* grown in separate culture

| Parameter | Parameter estimates (95% confidence interval) | |
|---------------|--|--|
| | Replication 1 | Replication 2 |
| g_{Lac} | 0.63 (0.53–0.73) | 0.70 (0.58–0.73) |
| Lac_{max_0} | 1.605×10^8 (6.054×10^7 – 2.605×10^8) | $3.162 \cdot 10^7$ (3.161×10^7 – 1.316×10^8) |
| β_{Lac} | 0.23 (0.12–0.32) | 0.25 (0.15–0.35) |
| k_{21} | 2.615×10^{-8} (6.146×10^{-9} – 4.617×10^{-8}) | 5.020×10^{-8} (3.020×10^{-8} – 7.019×10^{-8}) |
| k_{22} | 0.099 (0.080–0.120) | 0.123 (0.103–0.143) |
| p_1 | 2.79×10^{-5} (1.90×10^{-5} – 3.57×10^{-5}) | 3.45×10^{-5} (2.54×10^{-5} – 4.32×10^{-5}) |
| p_2 | 1.87×10^{-6} (1.22×10^{-6} – 2.63×10^{-6}) | 4.42×10^{-6} (3.39×10^{-6} – 5.67×10^{-6}) |
| κ | 0.24 (0.17–0.33) | 0.24 (0.24–0.26) |

Table 3. Conditional estimates and 95% confidence intervals of the interaction parameters for *Listeria monocytogenes* and *Lactobacillus sake* grown in mixed culture. Estimated parameters are λ_{lis} , λ_{lac} and ν , other parameters are fixed at the values given

| Parameter | <i>L. monocytogenes</i> | <i>Lact. sake</i> |
|-------------------------------|---|---|
| g_{Lis}/g_{Lac} | 0.40 | 0.62 |
| Lis_{max_0}/Lac_{max_0} | 3×10^8 | 1×10^8 |
| β_{Lis}/β_{Lac} | 0.1 | 0.2 |
| k_{11}/k_{21} | 7.5×10^{-9} | 2.7×10^{-8} |
| k_{12}/k_{22} | 0.03 | 0.095 |
| p_1 | | 2.5×10^{-5} |
| p_2 | | 2×10^{-6} |
| $\lambda_{Lis}/\lambda_{Lac}$ | 4.23×10^{-7} (2.31×10^{-8} – 1.43×10^{-6}) | 4.77×10^{-6} (7.89×10^{-7} – 1.48×10^{-5}) |
| ν | | 0.65 (0.42–0.87) |
| κ | | 0.24 |

decline in *Listeria* numbers in the mixed culture is due to the effect of bacteriocin.

The proposed model describes microbial growth in a quite general way. The model employs the commonly used Logistic growth law for the populations of *L. monocytogenes* and *Lact. sake*. The model also includes the effect of the micro-organism’s metabolism in converting nutrients into by-products which alter the environment, in this case pH of the growth medium. Interaction terms include different possible mechanisms of mutual influence of both populations in mixed culture. This complex of the interactions is a novel feature of the new model.

The models for the growth of the *L. monocytogenes* (equation 1) and for the *Lact. sake* populations (equation 2) both show a good fit to the data. There was no significant difference between the parameter estimates in each of the

two replications, with the exception of the parameter associated with the relationship between bacteriocin production and *Lact. sake* population size. In this case the parameter value in replication 2 was larger ($P < 0.05$) than that in replication 1.

Listeria monocytogenes had a faster ($P < 0.01$) population-specific growth rate than *Lact. sake* and a higher estimated maximum population size when each species was grown separately. However, the differences in the maximum population size reached significance ($P < 0.05$) for only replication 2 of *Lact. sake*. In both species the change in pH was more affected by the number of microbes present rather than the rate of the population change.

Lactobacillus sake generated a lower pH at a faster rate ($P < 0.05$) than *L. monocytogenes* (parameters k_{11} and k_{12}). This is not surprising because *L. sake* produces mostly lactic acid

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which affects pH more than the mix of organic acids produced by *L. monocytogenes*. This was also observed by Breidt and Fleming (1998) in a study of acid production by *Lactococcus lactis* and *L. monocytogenes*. However, their experiments showed pH change to be the main mechanism for *L. monocytogenes* growth suppression and not bacteriocin production, which is the mode indicated by the present analysis of a *L. monocytogenes*–*Lact. sake* model.

Bacteriocin is most actively produced by *Lact. sake* during the exponential phase of growth. In previous studies Nielsen et al. (1990), using *Pediococcus acidilactici*, and Hugas et al. (1995), using *Lact. sake*, observed that both population growth and size affected the production of bacteriocin. More recently, Leroy and De Vuyst (1999) proposed a model of bacteriocin production in *Lact. sake* in which production was driven by population growth alone, and reported good agreement with data. However, the biokinetic parameters in their study were manipulated until a good visual fit of the curves was obtained. This is in some agreement with our observation of the dominant role of the phase of population growth of *Lact. sake*, although proper statistical procedure of data fitting shows that the population size itself is also of importance. That is, our analysis shows that both the population growth rate and some non-growth aspects of population size should be taken into account to describe the bacteriocin production.

The modelling of population growth for each species grown in mixed culture (equations 3) also shows good agreement with experimental observations. The parameters determined from experimental data from single species cultures are applicable to the mixed species case. This is natural for the chosen structure of the model. If, in these equations, all interactions between cultures are ignored, the equations will be divided into independent sets, (1) and (2), for *L. monocytogenes* and the *Lact. sake*, respectively.

This feature allows the use of parameter estimates from the single cultures for calculating the parameters of interaction in the mixed system. In other words, the parameters in the mixed culture can be estimated conditional on the values of the parameters for the non-interacting cultures.

The major effect of bacteriocin on the *L. monocytogenes* population is clear, and it appears that the detrimental effects of lower pH, or interaction of microbes on growth rates, is not responsible for the observed fast elimination of *L. monocytogenes* in the presence of a source of bacteriocin.

The actual biokinetics taking place for the mixed culture described are likely to be more complicated than those considered by the described model. However, the fit of the experimental data by equations (1–3) suggests that the proposed model provides a reasonable description of the mixed population growth of these two microbes.

There is considerable interest in the manipulation of bacteriocin on food safety (Post 1996). One approach is to introduce a microbe such as *Lact. sake* into food to inhibit the growth of pathogens such as *L. monocytogenes*. The management of this process is a challenge, especially on an industrial scale. The development of models of the microbial population interactions will assist this management. This will require the extension of models such as equation (3) to deal with the variable external conditions, such as a changing temperature. However, these issues can be modelled within the differential equation framework adopted here.

The model constructed in this paper produces all the qualitative behaviour observed in previous experiments on microbial interactions involving bacteriocins (Leroy and de Vuyst 1999, Lejeune et al. 1998). The model can also reproduce all the experimental results observed by Winkowski and Montville (1992) for *Lactobacillus bavaricus* and *L. monocytogenes* growing together. This suggests that the generic model correctly describes the process of interaction between the microbial populations that involves bacteriocin, although naturally the parameter values will be different in this case.

The statistical analysis of the model gives estimates of the bacteriocin decay rate (κ), the bacteriocin production rate associated with both population growth and size, and the rates of elimination of *L. monocytogenes* for a given amount of bacteriocin (ν). The estimates of these effects made in a dynamic system are a useful reference for measurements made in

controlled experiments that may not have the benefit of experiencing the population interactions.

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Queries and / or remarks

| Manuscript Page/line | Details required | Author's response |
|-------------------------|---|-------------------|
| Page 1 | Please provide received date | |
| Page 5 line 6 | 6N NaOH should this be molar? | |
| Page 6 line 7 | Equations 2 and 3 mention the variable <i>b</i> . This is not described in the paragraph at the bottom of p 7. Could you add a sentence to do so. | |
| Page 11 line 6 | The first para in the results section indicates that <i>L. Monocytogenes</i> is producing bacteriocin. Is this correct? | |