Prediction of Microbial Growth in Mixed Culture with a Competition Model

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Prediction of microbial growth in mixed culture was studied with a competition model that we had developed recently. The model, which is composed of the new logistic model and the Lotka-Volterra model, is shown to successfully describe the microbial growth of two species in mixed culture using Staphylococcus aureus, Escherichia coli, and Salmonella. With the parameter values of the model obtained from the experimental data on monoculture and mixed culture with two species, it then succeeded in predicting the simultaneous growth of the three species in mixed culture inoculated with various cell concentrations. To our knowledge, it is the first time for a prediction model for multiple (three) microbial species to be reported. The model, which is not built on any premise for specific microorganisms, may become a basic competition model for microorganisms in food and food materials.

Key words: Prediction / Microbial growth / Mixed culture / Competition model / Logistic model.

Fresh food and food materials are often contaminated with natural microflora (NM) from the soil, the sea, and domestic animals, and are thought to be ecosystems for microbes. Thus, when a species of concern enters food and food materials already contaminated with NM, it would be too complex to describe and predict the growth of the species of concern and others in the food using mathematical models.

Many researchers have studied the growth of a food-borne pathogen competing with NM in food using mathematical models (Breidit and Fleming, 1998; Gimenez and Dalgaard, 2004; Le Marc et al., 2009; Koseki et al., 2011). We also successfully predicted Salmonella growth in raw ground chicken and liquid egg products with our growth model and the maximum population kinetics of the organism (Zaher and Fujikawa, 2011, Sakha and Fujikawa, 2012 and 2013). This is practically beneficial, but not based on a basic competition model or system. Thus, we wished to develop a new, basic competition model (or system) using our model.

A basic mathematical model for the growth of competing microbes would be a useful tool to study the growth kinetics of the contaminants in food. In order to build such a basic model, we need to start with the competition between two species, the simplest type. Once a basic model for two competitors is established, the model could be further developed for application to the competition among multiple species.

Recently we studied microbial growth in mixed culture with a competition model (Fujikawa et al., 2014). As a result, we found that a model composed of the new logistic (NL) model and the Lotka-Volterra (LV), a very well-known and general model for the description of competition between two species in ecology, successfully described the growth of two species in mixed culture with Staphylococcus aureus, Escherichia coli, and Salmonella. We called the new competition model the NL-LV model. When the Baranyi model was also examined for comparison, the combination with the Gimenez and Dalgaard (GD) model (2004) described the growth in mixed culture well.

Thus, for the next step in the evaluation of this model,
we needed to study the ability of the NL-LV model to predict microbial growth in mixed culture. Here the parameter values in the model had to be evaluated before prediction and that the number of microbial species studied had to be three or more, because we had already studied the microbial growth in mixed culture of two competitors with our model (Fujikawa et al., 2014). Therefore, in the present study we studied the ability of the competition model to predict the simultaneous growth of three microbial species in mixed culture. To our knowledge, there have been no published papers to date on competition models capable of predicting the growth of three or more microbial species.

Microbial cells and experimental methods in the present study were the same as those in our previous study (Fujikawa et al., 2014). Namely, we inoculated bacterial cells of E. coli 1952, Salmonella Enteritidis 04-137, and S. aureus 10008 at various initial concentrations between 10^3 and 10^5 CFU/g into sterile ground chicken and stored the inoculated samples at a constant temperature (28°C). After various storage periods, samples were taken and then measured for the cell concentrations of the three species with selective agar plates. Each experiment was performed in triplicate and the averages for the populations of microbial species were then calculated at the data points.

The competition model for three species was developed with the NL and LV models on the basis of our previous study with two competitors (Fujikawa et al., 2014), as shown below.

\[
\frac{dN_i}{dt} = r_i N_i [1 - (N_i/N_{imax})^m] [1 - (N_{imax}/N_{in})^n] (1 - \frac{N_i^{C_1} + N_i^{C_2} + N_i^{C_3}}{N_{imax}}) \\
\frac{dN_j}{dt} = r_j N_j [1 - (N_j/N_{imax})^m] [1 - (N_{imax}/N_{in})^n] (1 - \frac{N_j^{C_1} + N_j^{C_2} + N_j^{C_3}}{N_{imax}}) \\
\frac{dN_k}{dt} = r_k N_k [1 - (N_k/N_{imax})^m] [1 - (N_{imax}/N_{in})^n] (1 - \frac{N_k^{C_1} + N_k^{C_2} + N_k^{C_3}}{N_{imax}})
\]

(1A,B,C)

Here \(N_i\) is the cell population of species \(i\) at time \(t\), \(r_i\), \(N_{imax}\), \(N_{in}\), and \(c\) are the rate constant of growth (or the maximum specific growth rate), the maximum population, the initial population, and the competition coefficient for species \(i\). \(m_i\) and \(n_i\) are the parameters for the curvature of the deceleration phase the period of the lag phase, respectively, for species \(i\).

The value of \(N_{imax}\) in Eq. 1 was expressed as the maximum value among the three species (Eq. 2).

\[N_{imax} = \max\{N_{1max}, N_{2max}, N_{3max}\}\]

(2)

Eq. 1 was numerically solved with the 4th-order Runge-Kutta method in Microsoft Excel. The square root of the mean of the square error, \(RMSE\), between log-transformed cell concentrations estimated with the model and observed for the whole set of observation points, was calculated to evaluate the fitness of the model (Fujikawa et al., 2014).

For prediction with Eq. 1, the parameters other than the competition coefficients were estimated from the analysis of the monocultures, as we had analyzed for the two-species competition (Fujikawa et al., 2014). Competition coefficients for the three species were estimated as the averages from all the data of the two

![Graph A](image1.png)

![Graph B](image2.png)

![Graph C](image3.png)

**FIG. 1.** Prediction of the growth of the three species in mixed culture with the NL-LV model. The microorganisms were spiked at various initial concentrations as shown in A, B, and C. Symbols: ○, S. aureus; □, Salmonella; △, E. coli. Bars show the SDs. Solid lines are predicted with the model.
competitive species studied; the values were 1.03 for Salmonella, 1.04 for E. coli, and 1 for S. aureus. Here the value for S. aureus was fixed to 1 at unity for comparison among the species.

The competition model successfully predicted the growth curves of the three species in mixed culture at various initial concentrations, as shown in Fig. 1. The averages of RMSE in the population in Fig. 1 were very small, being A. 0.250, B. 0.269, and C. 0.255 in log. The average for the three was 0.258±0.0101 in log. Among the monoculture of these strains, E. coli grew the fastest, followed by Salmonella and S. aureus in order (Fujikawa et al., 2014). It was shown that these growth potentials of the strains dominated the growth of the species in mixed culture at the various initial cell concentrations as well. This was also shown by the prediction with our model.

The residuals between the observed and estimated populations for the bacterial species were then analyzed along with time. Here the residual was the value of the measured cell concentration (log) minus the predicted cell concentration (log) for each observation point during the growth. In terms of the rates of acceptable values between +0.5 and -1.0 log, 93.7% (59/63) of all the points were located in this range, which was greatly over the level of acceptance (70%) (Oscar, 2009) (Fig. 2). When the acceptable range was set between +0.5 and -0.5 log, 92.1% (58/63) were still in this range. The average of the residuals for all points was small, being 0.109 in log.

These results suggested that the present model was suitable for predicting the growth of the three species in the mixed culture. The previous and present studies (Fujikawa et al., 2014) showed that the NL-LV model could be a basic model for microbial growth in mixed culture.

There were two candidates for the $N_{\text{max}}$ value in the LV term in the NL-LV model, namely, the dominant type and the sum type. There was no significant difference in the $RMSE$ for the growth of the two competitors between the two candidates by the $t$-test ($p=0.94$) (Fujikawa et al., 2014). In the present study, the average of $RMSE$ for the growth in Fig. 1 with the dominant type $(0.258\pm0.0101$ in log) was close to that with the sum type $(0.264\pm0.0468$ in log). More studies will be needed to make a decision on the two candidates.

For the growth of the three species in mixed culture, we could get growth curves closer to the observed populations than the predicted curves, by optimizing the values for the competition coefficients of the NL-LV model to the data with the minimum $RMSE$ value. The optimization was done with the Solver function in Excel. For example, the $RMSE$ values obtained by the optimization for the growth in Fig. 1 were obtained as A. 0.181, B. 0.241, and C. 0.224 in log; the average for the three was 0.216±0.0312 in log. This was slightly smaller than those by prediction $(0.258\pm0.0101$ in log). However, this is curve fitting. Prediction with a model has to be done with the parameter values of the model previously obtained by data analysis.

Since we showed that the NL-LV model could well describe and predict microbial competitive growth for up to three species in mixed culture, we could make a general form of the model. The general form of our model would be expressed as Eq. 3 for species $i$ in a microbial community consisting of a total of $p$ species. Here $i$ and $p$ ($>1$) are natural numbers.

$$\frac{dN_i}{dt} = r_iN_i \left[1 - \left(\frac{N_i}{N_{i\max}}\right)^n\right] \left[1 - \left(\frac{N_{i\max}}{N_i}\right)^m\right] \left(1 - \frac{\sum_{i=1}^{p} N_i^n}{N_{i\max}}\right)$$

For $N_{i\max}$ in Eq.3, the two candidates of the dominant type and the sum type could be considered, as discussed above.

In this study, we showed a new microbial competition model with the NL model. Now we are further studying the growth prediction in mixed culture at dynamic temperature patterns using the three species studied here. Namely, we are studying the growth kinetics of each mono-culture at constant temperatures and then predicting the growth of each species in mixed culture at dynamic temperature patterns with the present competition model, as we successfully predicted for single species (Fujikawa et al. 2003, 2004; Fujikawa and Morozumi, 2005).

To our knowledge, there have been no published papers to date on competition models capable of

![Fig. 2](image-url)
predicting the growth of three or more microbial species, as described above. We think that the present model, which is not built on any premise for specific microorganisms, might become a basic competition model for microorganisms in food and its materials.

Since the Baranyi-GD model could well describe the microbial growth of two competitors (Fujikawa et al., 2014), the Baranyi-GD model for three species was developed in the present study, as shown below.

$$\frac{dN_i}{dt} = \mu_{max} Q_i N_i \left[ 1 - \left( \frac{N_i}{N_{max}} \right)^a \right] \left[ 1 - \left( \frac{N_j}{N_{max}} \right)^b \right] \left[ 1 - \left( \frac{N_k}{N_{max}} \right)^c \right]$$

$$\frac{dN_j}{dt} = \mu_{max} Q_j N_j \left[ 1 - \left( \frac{N_j}{N_{max}} \right)^a \right] \left[ 1 - \left( \frac{N_k}{N_{max}} \right)^b \right] \left[ 1 - \left( \frac{N_i}{N_{max}} \right)^c \right]$$

$$\frac{dN_k}{dt} = \mu_{max} Q_k N_k \left[ 1 - \left( \frac{N_k}{N_{max}} \right)^a \right] \left[ 1 - \left( \frac{N_i}{N_{max}} \right)^b \right] \left[ 1 - \left( \frac{N_j}{N_{max}} \right)^c \right]$$

Here $\mu_{max}$ and $Q$ are the maximum specific growth rate and the concentration of the biomolecules in the microbial cells that regulate the proliferation of cell for species $i$. $l_{ij}$ is the competition coefficient for species $j$ to $i$.

Prediction with this model was done in the same manner as we did for the NL-LV model. Namely, the averages for the parameters of the Baranyi model for the monoculture and those for the competition coefficients with the two competitors obtained in our recent study (Fujikawa et al., 2014) were input into Eq. 3 for prediction. Eq. 3 was then numerically solved with the 4th-order Runge-Kutta method in Microsoft Excel.

Consequently, this model could not well predict microbial growth in mixed culture with the three species. One example is shown in Fig. 3, which corresponds to Fig. 1A with the NL-LV model. The predominant species predicted among the three species (Salmonella) was different from that measured experimentally (E. coli). The RMSE value for this example was as high as 0.584 in log, which was 2.3 times higher than that with the NL-LV model (0.250). The reason for the poor prediction results was not understood.

**REFERENCES**


