Development of a Competition Model for Microbial Growth in Mixed Culture

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A novel competition model for describing bacterial growth in mixed culture was developed in this study. Several model candidates were made with our logistic growth model that precisely describes the growth of a monoculture of bacteria. These candidates were then evaluated for the usefulness in describing growth of two competing species in mixed culture using *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella*. Bacterial cells of two species grew at initial doses of $10^3$, $10^4$, and $10^5$ CFU/g at $28^\circ$C. Among the candidates, a model where the Lotka-Volterra model, a general competition model in ecology, was incorporated as a new term in our growth model was the best for describing all types of growth of two competitors in mixed culture. Moreover, the values for the competition coefficient in the model were stable at various combinations of the initial populations of the species. The Baranyi model could also successfully describe the above types of growth in mixed culture when it was coupled with the Gimenez and Dalgaard model. However, the values for the competition coefficients in the competition model varied with the conditions. The present study suggested that our model could be a basic model for describing microbial competition.

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\[ \frac{dN}{dt} = rN\{1-\left(\frac{N}{N_{\text{max}}}\right)^m\}\{1-\left(\frac{N}{N_{\text{min}}}\right)^n\} \]  

Here $N$ is the microbial population at time $t$. $N_{\text{max}}$ and $N_{\text{min}}$ correspond to the maximum and the initial cell populations, respectively. $m$ and $n$ are the parameters for the curvature of the deceleration phase and the period of the lag phase, respectively (Fujikawa, 2010).

The NL model has been shown to describe and predict microbial growth in broth and on agar plates successfully at constant and dynamic temperatures (Fujikawa et al., 2003 and 2004; Fujikawa and Morozumi, 2005). It could further predict the amount of *staphylococcal enterotoxin A* produced by *Staphylococcus aureus* in milk and a blue pigment caused by *Pantoea agglomerans* on agar plate (Fujikawa and Morozumi, 2006; Fujikawa and Akimoto, 2011). We now consider the NL model to be a...
phenomenological or descriptive model for microbial growth, because a biological interpretation in the lag term in the model has yet to be found. There are several relationships among microbial species in the environment, including mutualism, competition, commensalism, and amensalism (Bailey and Ollis, 1986). Among these relationships, competition would be the most common for interacting microbial species, because all microbial species need nutrients and space for growth. In competition, the consumption of a common factor such as food supply by each species limits its availability to others, so the growth rates of the interacting organisms are affected negatively (Bailey and Ollis, 1986).

Fresh food and food materials such as vegetables, fish, and meat, which are often contaminated with natural microflora from the soil, the sea, and domestic animals, are thought to be ecosystems for microbes. Thus, when a species of concern such as Salmonella contaminates food containing other non-harmful microorganisms, it would be too complex to describe and predict the growth of the species of concern and others in the food using mathematical models. 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 to apply to competition among multiple species.

Many researchers have studied the growth of a foodborne pathogen competing with the natural microflora in food using mathematical models. Breidt and Fleming (1998) modeled the competitive growth of Listeria monocytogenes and Lactococcus lactis by modeling the concentration of lactic acid produced and the pH of vegetable broth. Gimenez and Dalgaard (2004) also developed a competition model for L. monocytogenes and lactic acid bacteria, which was based on the logistic model. This model requires the separate estimation of the period of the lag phase, but the term of suppression in the model successfully described the growth curves of these microorganisms. Le Marc et al. (2009) developed a competition model by introducing the concept of critical population density for lactic acid bacteria against a competing bacterium (S. aureus). These models do seem to be specific to the species of concern, but not general enough to apply to a number of microbial species. 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 needed to develop a new, basic competition model using our model.

The Lotka-Volterra (LV) model is a very well-known and general model for the expression of competition between two species in ecology (Vandermeer and Goldber, 2003). When species 1 grows in competition with species 2 in a given environment, the LV model is expressed by the following function.

\[ f(N_1, N_2) = \frac{N_1 + N_2}{N_{max}} \]

\[ N_1 \text{ and } N_2 \text{ are the populations for the corresponding species at a given time. Here } N_{max} \text{ can be set as a larger value between the maximum populations of the two species, as shown below.} \]

\[ N_{max} = \max(N_{1max}, N_{2max}) \]

As the sum of the two populations increases with time to the value of \( N_{max} \) in Eq. 2, the value for the function approaches one, leading to no increase in the populations when the model is incorporated in a growth model. Many researchers have introduced the LV model into the original logistic model in mathematical ecology (Vandermeer and Goldber, 2003).

Dens et al. (1999) proposed a microbial competition model constructed with the Baranyi model and the LV model and showed the characteristics of the competition model. However, since their studies were mathematical simulations, one needs to evaluate whether their model is applicable to real microbial competition with microbial data. Similar studies were also reported by other researchers (Powell et al., 2004; Vereecken et al., 2000). Liu et al. (2006) studied interactions of microorganisms on pork with the modified Gompertz model coupled with a modified LV model. They showed the parameter values of microbial growth in their model, but not the actual growth curves. Thus, one cannot evaluate how accurately their model describes competitive growth.

Under these circumstances in modeling microbial growth in food and food material, the aim of the present study was to develop a basic competition model for multiple (more than two) species in mixed culture. In this study, therefore, we developed new models for the competitive growth of microbial species by introducing the LV model into the NL model, and these models were then validated using microbial data. The introduction of the Gimenez and Dalgaard (GD) model (2004) into the NL model was also studied. We here used three microbial species, non-pathogenic
Escherichia coli, Salmonella Enteritidis, and S. aureus, as competitors, because the measurement of populations of these species has been well established by using good selective agar media. In this study, thus, there were no problems in terms of whether the test strains were pathogens or non-pathogens and spoilage bacteria or non-spoilage bacteria from the viewpoint of modeling microbial competition. Various combinations of the initial populations of the test strains were examined to evaluate the competition models. Since the test strains were all mesophiles, we examined the growth of these strains at a moderate temperature (28°C). We here selected sterilized ground chicken as the solid culture medium for study, because the culture medium used in this study needed to be homogeneous in order to obtain quantitative data of microbial populations. Consequently we could obtain a model suitable for describing the growth of two species in mixed culture.

**MATERIALS AND METHODS**

**Bacterial cell preparation**

Bacterial strains of *E. coli* 1952 (Fujikawa et al., 2003 and 2004; Fujikawa and Morozumi, 2005), *Salmonella* Enteritidis 04-137 (Zaher and Fujikawa, 2011), and *S. aureus* 10008, which produced staphylococcal enterotoxin A (SEA), were used for study. The *E. coli* and staphylococcal strains were isolates from commercial food products in Tokyo, Japan. The *Salmonella* strain was an isolate from a *Salmonella* outbreak in Tokyo. Bacterial cell suspensions of each strain were prepared by our method (Zaher and Fujikawa, 2011). Briefly, cells of the strain, which had been kept in semi-solid agar medium, were activated on a nutrient agar plate (Nissui Pharmaceuticals Co., Ltd., Tokyo, Japan) or a selective agar plate as described below at 37°C for 24 h or 48 h (for *S. aureus* only). Cells of several well-grown colonies on the plate were used for incubation in trypticase soy broth (Oxoid, Basingstoke, England) with shaking at 37 °C and 110 rpm for 24 h. Cultured cells were washed by centrifugation and then serially diluted with saline (0.85% (w/v) sodium chloride solution), in order to obtain cell concentrations of 10^5, 10^4, and 10^3 CFU/ml.

**Spiking cells and storage**

Ground chicken breast was purchased at a retail store in Tokyo and put into sterile plastic cups. The ground chicken was frozen at -20°C until use and then thawed at <10 °C overnight for use. The chicken was sterilized at 121°C for 15 min and then cooled at room temperature. The sterilization of the chicken was confirmed by the standard agar plate method (Anonymous, 2004).

The culture medium (the sterilized chicken) was spiked with two microbial cell suspensions prepared as described above (each 2 ml per 100 g of chicken). Combinations of the initial populations for the two species were (i) 10^3 versus 10^5 CFU/g, (ii) 10^4 versus 10^6 CFU/g, and (iii) 10^5 versus 10^7 CFU/g. As a monoculture sample, the control was also spiked with a single cell suspension at 10^3, 10^4, or 10^5 CFU/g and saline. After thorough mixing, 10-g portions of the culture medium sample were placed in sterile glass bottles (110 ml vacant volume) with caps (Zaher and Fujikawa, 2011).

The glass bottles were stored in an incubator (MIR-154; Sanyo, Tokyo, Japan) at 28°C. The time required in order for the sample in the bottle to reach the designated temperature in the incubator was measured with a digital thermometer (AM-7002; Anritsu Meter Co., Tokyo) and was taken into consideration during the experiment (Fujikawa et al., 2003 and 2004; Fujikawa and Morozumi, 2005). Immediately after each storage period, the sample (one bottle per point) was taken from the incubator and cooled in ice water. Each experiment was performed in triplicate.

**Bacterial cell counts**

The samples were mixed with buffered sodium chloride peptone solution (90 mL) (Nissui Pharmaceuticals) to make 10% food homogenate and then transferred to a sterile filtered plastic bag. The homogenate was thoroughly mixed in a stomacher (SH-111; Elmex, Tokyo) for one minute. Each sample was then serially 10-fold diluted with saline (Anonymous, 2004). Diluted samples (0.1 mL each) were plated on selective agar plates in duplicate. Xylose lysine deoxycholate (XLD) agar plates (Oxoid) were used for the enumeration of *Salmonella*, CHROMagar *E. coli* (CHROMagar, Paris, France) for *E. coli*, and Baird-Parker agar plates (Oxoid) for *S. aureus*. After incubation following the instructions for the corresponding selective agar plates, bacterial colonies on agar plates for the samples were counted. For each storage condition, counts for two plates were taken into account, and the averages and standard deviations (SD) for three trials per observation point were then calculated.

**Growth analysis**

Growth data of the monoculture (the control) for test microorganisms were analyzed using a computer program based on the NL model to estimate the values for *r*, *m*, and *n* in Eq. 1 (Fujikawa and Kano, 2009). Here *r* was estimated from the slope of an experimental
growth curve.

Growth data were also analyzed with DMFit, a Microsoft Excel add-in software (kindly provided by Dr. J. Baranyi, Institute of Food Research, Norwich, UK) and the parameters including \( \mu_{\text{max}}, \text{lag}, \gamma'(0) \), and \( \gamma'(\text{end}) \) of the Baranyi model were estimated. Here the values for \( m_{\text{Curv}} \) and \( h_0 \) in the DMFit were set to both 10 (the default).

**Development of competition models**

Several competition models were developed by introducing the LV model into the NL model. First, a model was developed in which the LV model (Eq. 2) was directly used in the term having \( N_{\text{max}} \) of the NL model (Eq. 1) was developed. We call it the original LV model. Competition coefficients \( c_1 \) and \( c_2 \) were also introduced to express the relative strengths of growth between two competing species (Vandermeer and Goldber, 2003). There were considered to be two types in terms of the competition coefficient, namely a multiplication type (type M) and a power type (type P), as shown in Eq. 4A, B and 5A, B, respectively (Table 1).

Secondly, a model was created in which the LV model was introduced as an additional term in the NL model; we call it the LV-extended model. There are also two types of this model according to the positions of the competition coefficients, that is, a multiplication type (type M) and a power type (type P), as shown in Eqs. 6A, B and 7A, B, respectively (Table 1).

Thirdly, the GD model (Gimenez and Dalgaard, 2004) was introduced in the NL model instead of the LV model (Eqs. 8A, B) (Table 1). Here \( l_{11} \) and \( l_{12} \) are competition coefficients of species 2 for species 1 and of species 1 for species 2, respectively.

All models examined in the present study were numerically solved with the 4th-order Runge-Kutta method in Microsoft Excel (Fujikawa et al., 2003 and 2004; Fujikawa and Morozumi, 2005; Fujikawa and Kano, 2009).

The square root of the mean of the square error, RMSE, between log-transformed cell concentrations

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**TABLE 1.** Competition models proposed in the present study.

<table>
<thead>
<tr>
<th>Model Type</th>
<th>Model Equation</th>
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| A. NL model coupled with original LV model (type M) | \[
\frac{dN_1}{dt} = r N_1 \left[ 1 - \left( \frac{c_1 N_1 + c_2 N_2}{N_{\text{max}}} \right)^m \right] \left[ 1 - \left( \frac{N_{\text{min}}}{N_1} \right)^n \right]
\]
| B. NL model coupled with original LV model (type P) | \[
\frac{dN_1}{dt} = r N_1 \left[ 1 - \left( \frac{N_{\text{max}}}{N_1} \right)^m \right] \left[ 1 - \left( \frac{N_{\text{min}}}{N_1} \right)^n \right]
\]
| C. LV-extended NL model (type M) | \[
\frac{dN_1}{dt} = r N_1 \left[ 1 - \left( \frac{N_{\text{max}}}{N_1} \right)^m \right] \left[ 1 - \left( \frac{N_{\text{min}}}{N_1} \right)^n \right] \left( 1 - \frac{c_1 N_1 + c_2 N_2}{N_{\text{max}}} \right)
\]
| D. LV-extended NL model (type P) | \[
\frac{dN_1}{dt} = r N_1 \left[ 1 - \left( \frac{N_{\text{max}}}{N_1} \right)^m \right] \left[ 1 - \left( \frac{N_{\text{min}}}{N_1} \right)^n \right] \left( 1 - \frac{c_1 N_1 + c_2 N_2}{N_{\text{max}}} \right)
\]
| E. NL model coupled with the Gimenez and Dalgaard (GD) model | \[
\frac{dN_1}{dt} = r N_1 \left[ 1 - \left( \frac{N_{\text{max}}}{N_1} \right)^m \right] \left[ 1 - \left( \frac{N_{\text{min}}}{N_1} \right)^n \right] \left( 1 - \frac{c_1 N_1 + c_2 N_2}{N_{\text{max}}} \right)
\]
estimated with the models \(N_{\text{est}}\) and observed \(N_{\text{obs}}\) for the whole set of observation points \(k\), as shown below, was calculated to evaluate the fitness of the models.

\[
RMSE = \sqrt{\frac{1}{k} \sum_{i=1}^{k} (\log N_{\text{obs}} - \log N_{\text{est}})^2} \tag{9}
\]

Values for the parameters and the competition coefficients in the developed models (Table 1) were evaluated by minimizing \(RMSE\) for each experiment, which was carried out with the Solver function in Microsoft Excel.

**Evaluation of model performance**

Performance of the models in the present study was evaluated with the \(RMSE\) value and the residual, which is the value of \(\log N_{\text{obs}}\) minus \(\log N_{\text{est}}\), for each observation point during the growth (Oscar, 2009). The Akaike’s Information Criterion (\(AIC\)) (Akaike, 1973) was also used for model evaluation. The \(AIC\) value for a model was obtained with the \(RMSE\) value and the number of the parameters in the model, as shown below.

\[
AIC = (k+1)\ln\left(\frac{2\pi}{k}\right) \left\{ \sum_{i=1}^{k} (\log N_{\text{obs}} - \log N_{\text{est}})^2 \right\} + 2 \times pm \tag{10}
\]

Here \(\pi\) is the circular constant and \(pm\) is the number of the parameters in the model. Statistical analysis was carried out with Microsoft Excel.

**RESULTS**

**Growth of two species in mixed culture**

The growth of two microbial species in mixed culture at various combinations of the initial concentrations of \(10^3\), \(10^4\), and \(10^5\) CFU/g was studied for \(S.\) aureus, Salmonella, and \(E.\) coli. Growth curves of \(S.\) aureus and Salmonella in the mixed culture and the monocultures are shown as examples in Fig. 1. In this example, the smaller the initial population, the greater the suppression was observed for \(S.\) aureus in the mixed culture (Fig. 1A), whereas very weak suppression of Salmonella growth was observed in the mixed culture (Fig. 1C).

The growth curves of monoculture for the bacterial species were then studied for description with the NL model. The model successfully described the growth of the monocultures of the three species. Examples for \(S.\) aureus and Salmonella are shown in Fig. 1. The \(RMSE\) values for the growth curves in Fig. 1A-C by the model were very small, namely \(0.0955 \pm 0.0349\) (in log) on the average.

**Description of competitive growth**

The competition models shown in Table 1 were examined for their ability to describe the microbial growth in the mixed culture studied above.

The performance of the competition models in Table 1 was studied using the values for \(r\), \(m\), and \(n\) of the growth models obtained for the monoculture that were analyzed with the NL model. The competition coefficients of the competition models were then evaluated by minimizing the \(RMSE\) value for competitive growth.

Among the competition models, the LV-extended model \(\text{(type P)}\) (Eqs. 7A, B) successfully described the growth curves of the two species in mixed culture;
species and also the averages of the coefficient were very close to one, ranging from 1.02 to 1.04. Also, the values for the coefficient of variance corresponded to that in Fig. 1. Symbols: very small Salmonella. Bars show the SDs. The values for the competition coefficient of type M did not vary, while those for type P were almost constant with an average of 1.03±0.0351 (Table 2). This result clarified that type P (Eqs. 7A, B) was mathematically a robust model.

The combination of the original LV models or the GD model with our growth model (Eqs. 4A, B, 5A, B, 8A, B) could not sufficiently describe the competitive growth curves. That is, these models showed higher RMSE and AIC values for growth curves than that of the LV-extended model (type P). For example, the averages for RMSE for the mixed culture of Salmonella and S. aureus (Fig. 2) by the original LV model types M and P were 0.399±0.427 and 0.365±0.370, which were 1.7 and 1.6 times higher than that by the LV-extended model (type P) (Table 2), respectively. The average for RMSE by the GD model was also high, being 0.734±0.262, which was 2.2 times higher. Also, the average for AIC for the mixed culture of Salmonella and S. aureus by LV-extended model (type P) (24.7±4.60) was smaller than those by the original LV model types M (26.7±7.48) and P (26.4±7.02), and the GD model (33.4±2.49).

On the basis of these results, we concluded that the LV-extended model (type P) was the best model for describing the growth of the two competitive species among the competition models developed in this study. Thus, this model was studied thereafter in the present study. Here we call it the NL-LV model.

The residuals between the observed and estimated populations (in log) for the points in the growth curves in Figs. 2-4 by the NL-LV model were analyzed along with time. The rate of acceptable values, namely, between +0.5 and -1.0 log, for all data points was 97.1% (134/138) for the model, which was considerably over the level of acceptance of 70% (Oscar 2009) (Fig. 5A). When a narrower acceptable range was set between +0.5 and -0.5 log, 95.7% (132/138) for the NL-LV model were still within this range (Fig. 5). The average of the residual for all points was -0.00443 (log). This value was very close to zero, showing no tendency to the positive or negative side of the residual. These results showed that the NL-LV model successfully described the competitive growth in Figs. 2-4.

**Comparison with the Baranyi model**

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Baranyi-GD models for the growth in Figs. 2-4 were 25.8 ± 3.61 and 27.0 ± 2.54, respectively. Similarly, there was not a significant difference between them according to Student’s t-test ($p=0.406$).

When the residuals by the Baranyi-GD model were plotted, 99.3% (137/138) of them were located between +0.5 and -1.0 log, and 94.9% (131/138) between +0.5 and -0.5 log, showing that the residuals by this model were also quite small (Fig. 5B). The averages for all growth in Figs. 2-4 for the NL-LV model and the Baranyi-GD were 0.213±0.0800 and 0.206±0.0630, respectively, which were both very small and close to each other. There was not a significant difference between them as analyzed by the with Student’s t-test ($p=0.890$).

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generally it is known that with more parameters in a mathematical model the fitting with the model becomes better. Thus, the AIC value, which incorporates the number of parameters in a model as well, is thought to be a good measure for the evaluation of the model (Akaike, 1973). As a result, the NL-LV model (type P) showed the lowest AIC value among the models in Table 1, showing that the model was the best among them.

The selective agar media for the bacterial cell measurements in the present study had to meet at least two requirements. First, the number of colonies on the selective agar plate for the test species needed to be the same as that on standard plate, control. Some selective agars give lower colony counts than the standard plate, selectivity. Second, the selective agar needed to easily differentiate colonies of the species of concern from other microbial colonies by colony characteristics like color and shape.

In a preliminary study, therefore, we chose the selective agar media suitable for the three microbes among several corresponding candidates. CHROMagar E. coli (CHROMagar), CHROMagar Orientation (CHROMagar), desoxycholate hydrogen sulfide lactose agar (Nissui) were tested for E. coli, mannitol salt agar.
Nissui, CHROMagar Orientation, Baird-Parker agar (Oxoid), and CHROMagar Staph aureus (CHROMagar) were tested for S. aureus. XLD (Oxoid), mannitol lysine crystal violet brilliant green agar (Oxoid), desoxycholate hydrogen sulfide lactose agar (Nissui), and CHROMagar Orientation were tested for Salmonella. Standard agar (Nissui) was used as the control for all the organisms. The agar candidates were tested for monoculture and mixed culture of the species studied, and then the selective media that were described in the Materials and Methods section were selected for the three species in this study.

Oscar (2009) studied Salmonella growth on chicken skin with a general regression neural network and Monte Carlo simulation model, which were not competition models. He reported that 89% of independent data were located in the acceptance zone for the residuals with the model. Koseki et al. (2011) studied the growth of L. monocytogenes and isolates from the natural flora in raw minced tuna using the Baranyi-GD model. They succeeded in describing the growth of L. monocytogenes and the species from the natural microflora at the temperatures tested, except for low temperatures. In the plot of residuals, 75.3% of the points predicted for L. monocytogenes and 80.9% for the isolates from the natural microflora were located in the acceptable range between +0.5 and -1.0 log.

FIG. 5. Residual plots for the populations of the bacterial species by (A) the NL-LV model and (B) the Baranyi-GD model along through the storage period. All residuals between observed and estimated populations shown in Figs. 2-4 are plotted. Dotted lines show the boundaries of acceptance.

| TABLE 3. Values for competition coefficients and RMSE of competitive growth data in Figs. 2-4 analyzed with the Baranyi-GD model. |
|----------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                     | Competition coefficient |
| Initial populations (CFU/g)       | Species 1 | Species 2 | Species 1 | Species 2 | RMSE |
|-----------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| S. aureus + S. aureus             | 0.208          | 0.123          | 0.163          | 0.187          | 0.140          |
| S. aureus + E. coli               | 0.112          | 0.272          | 0.168          | 0.261          | 0.187          |
| S. aureus + E. coli               | 0.161 ± 0.0480 | 0.188 ± 0.0765 | 0.206 ± 0.0630 |                |                |
| CV                                | 29.8           | 40.7           |                |                |                |
| E. coli + S. aureus + E. coli     | 0.158          | 0.136          | 0.120          | 0.209          | 0.223          |
| E. coli + S. aureus + E. coli     | 0.0865         | 0.249          | 0.209          | 0.241          | 0.208          |
| E. coli + S. aureus + E. coli     | 0.121 ± 0.0357 | 0.197 ± 0.0576 | 0.224 ± 0.00134 |                |                |
| CV                                | 29.5           | 29.2           |                |                |                |
| S. aureus + E. coli + E. coli     | 0.178          | 0.162          | 0.126          | 0.243          | 0.224          |
| S. aureus + E. coli + E. coli     | 0.102          | 0.324          | 0.243          | 0.272          | 0.179          |
| E. coli + S. aureus + E. coli     | 0.135 ± 0.0390 | 0.243 ± 0.0809 | 0.225 ± 0.0380 |                |                |
| CV                                | 28.9           | 33.3           |                |                |                |

Competition coefficients are dimensionless.

a. Data in Fig. 2.
b. Values show the coefficients of variation, CV (%).
c. Data in Fig. 3. d. Data in Fig. 4.
Although the percentage of data falling into the acceptance zone depends on the experimental conditions and the models used, the value (93.7%) for prediction obtained in the present study can be regarded as satisfactory compared with the above values. Since the raw tuna in their study (Koseki et al. 2011) had been originally contaminated with natural microflora (at low levels), the growth of a monoculture of \textit{L. monocytogenes} in the tuna, a control, could not be measured. On the other hand, the culture medium used for the present study (i.e., ground chicken) was sterile, so this might be related to the above difference.

There might be two candidates for the \( N_{\text{max}} \) value in the LV term in the NL-LV model (type P). Namely, one is the \( N_{\text{max}} \) value of the dominant species in mixed culture, which was studied in the present study such as Eq. 3. Another is the sum of the \( N_{\text{max}} \) values of each species in mixed culture. Here we call them the dominant type and the sum type in order. The sum type, which has a larger capacity for microbial population than the dominant type, means that there might be an ecological niche for microbial growth in mixed culture that is not found in monoculture. In a preliminary study, we compared the two types in terms of the \( \text{RMSE} \) values for the growth curves in mixed culture. There was no significant difference in the \( \text{RMSE} \) for the growth curves in Figs. 2-4 between the two types by \( t \)-test \((p=0.94)\); the averages of \( \text{RMSE} \) for the growth curves with the dominant and the sum types were 0.213±0.0800 and 0.216±0.0777 in log, respectively. More studies will be needed in order to reach a conclusion on the two candidates for the \( N_{\text{max}} \) value.

To our knowledge, there are no published papers on competition models capable of predicting the growth of three or more microbial species. Now we are further studying the prediction of the simultaneous growth of the three microbial species in mixed culture with the NL-LV model in order to evaluate it. If our model succeeds in this prediction, it can be a basic model for competition. The Baranyi-GD model, which also successfully described the competitive growth between two species in this study, will be also examined for prediction of such growth.

**REFERENCES**


