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Minireview

Development of a New Logistic Model for Microbial Growth in Foods

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Mathematical models are essentially needed to quantitatively predict microbial growth in food products during their production and distribution. Recently we developed a new logistic model for microbial growth. The model is an extended logistic model, which shows a sigmoid curve on a semi-log plot. The model could precisely describe and predict bacterial growth at constant and dynamic temperatures in broth, on nutrient agar plates, and in pouched food. Prediction results with our model were very similar to those with the Baranyi model, which is well known worldwide. The model also predicted the amount of metabolites (toxins) that would be produced by a microorganism. Namely, with the growth model and the kinetics of staphylococcal enterotoxin A production, the amount of the toxins produced by *Staphylococcus aureus* in milk was successfully predicted. Our model could be a tool in the alert system and the quantitative risk assessment of harmful microbes in food.

Key words : Growth kinetics / Logistic model / Predictive model / Microbial growth.

Introduction

Consumers are greatly concerned about food safety at present. It is of utmost importance to ensure the microbiological safety of food products during their production and distribution. When food products are exposed to abuse (high temperatures) during these processes, the microbial contaminants would grow. Once the contaminants grow in a food product, they would not decrease in number even if the food is later refrigerated. Moreover, if the contaminants include pathogens like *Salmonella, Staphylococcus aureus*, or Verotoxin-producing *Escherichia coli*, a food poisoning outbreak could occur. Even if there were no pathogens, food spoilage could occur with the increased number of contaminants. Thus, microbial growth in commercial food products should be estimated to ensure food safety before consumers eat them.

The Malthus model, an exponential equation, is the simplest growth model for the description of a population, but the population estimated with that model increases to infinity with time. On the other hand, the logistic model is known to be a useful growth model for a population (Eq. 1) (Pielou, 1969).

$$\frac{dN}{dt} = rN\{1-(\frac{N}{N_{max}})\} \quad (1)$$

Here N is the population at time t, r is the rate constant of growth, and N_{max} is the maximum population. It describes a sigmoidal curve, which successfully fits growth patterns of populations such as those of humans and animals (Pielou, 1969). However, as shown in Fig. 1, the model cannot describe a sigmoidal curve on a semi-log plot; it can only describe a curve without a lag phase on the plot. Microbial growth often shows a sigmoidal curve on the plot.

The disadvantage of this model has led to the development of new growth models in predictive food microbiology. A lot of models have been developed to describe microbial growth so far (McMeekin et al., 1993). Two of the models, a modified Gompertz model and the Baranyi model, are known worldwide

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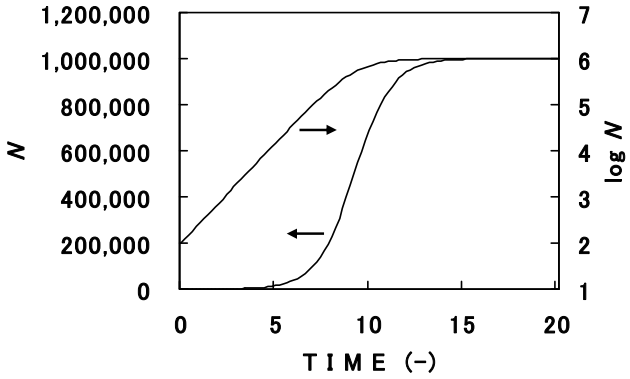

FIG. 1. Logistic curves plotted on the Cartesian and semi-log planes. Both curves are described by the logistic model with the same parameter values of $r=1$, $N_0=10^2$, and $N_{max}=10^6$. Arrows show the corresponding axes.

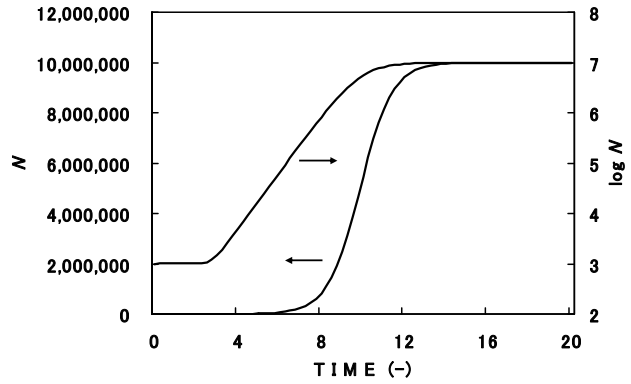

FIG. 2. New logistic curves plotted on the Cartesian and semi-log planes. Both curves are described with the same parameter values of $r=1.3$, $m=1$, $n=3.5$, $N_0=10^3$, and $N_{max}=10^7$. Arrows show the corresponding axes.

(Gibson et al., 1987; Baranyi and Roberts, 1995). The modified Gompertz model is merely an empirical model and cannot predict growth at varying temperatures. The Baranyi model shows good performance as shown below, but there is an essential problem in its concept of modeling microbial growth. That is, the model is built on the assumption that the concentration of a substance or substances critical to microbial growth (such as RNA molecules or ATP) would increase exponentially in a cell during the whole growth period (Baranyi and Roberts, 1995). However, no intracellular substances in a cell could increase exponentially to infinity during the growth period and there is always binary fission in the cell cycle. Furthermore, the model cannot describe a growth curve without a lag phase.

Thus, we tried to develop a new mathematical model and then made a model based on the logistic model (Fujikawa et al., 2003). It successfully described and further predicted microbial growth at various patterns of temperature (Fujikawa et al., 2004; Fujikawa and Morozumi, 2005; Fujikawa et al., 2006b).

Characteristics of the new logistic model.

After small modifications, the new logistic model is shown as follows (Fujikawa and Morozumi, 2005):

$$\frac{dN}{dt} = rN\{1-(\frac{N}{N_{max}})^m\}\{1-(\frac{N_{min}}{N})^n\} \qquad (2)$$

We introduced N_{min}, which is related to the initial population, N_0 in the model. N_{min} and N_{max} are asymptotes. N_{min} needs to be almost equal to and a bit smaller than N_0. Here we set N_{min} smaller than N_0 by one ppm portion of it, being expressed as $(1 - 1/10^6) \times N_0$. This reduction rate ($1/10^6$) practically produced the smallest differences between the calculated and measured cell populations (Fujikawa et al., 2003). m and n are parameters. We now call Eq. 2 a generic

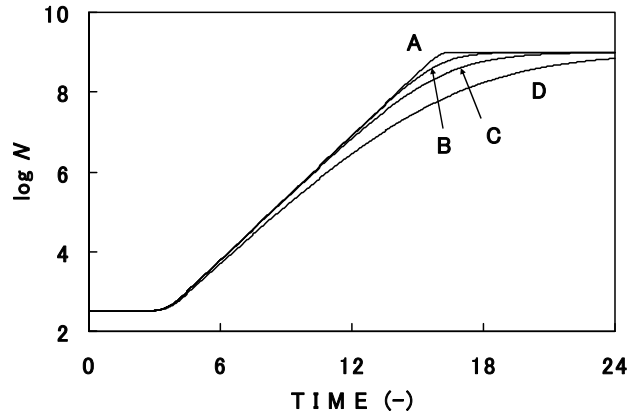

FIG. 3. Effect of parameter m on the new logistic curve. Here $r=1.2$, $n=3$. Curves are described with various values of m for A 5, B 1, C 0.5, and D 0.25.

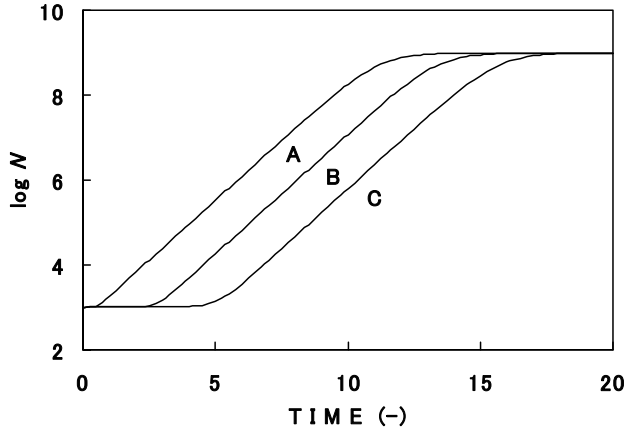

FIG. 4. Effect of parameter n on the new logistic curve. Here $r=1.3$, $m=1$. Curves are described with various values of n for A. 20, B. 3.5, and C. 2.5.

form of the model.

In the model, the population of a target microbe monotonously increases from N_{min} to N_{max} with time. The growth rate by the model (Eq. 2) at the beginning is suppressed by the term of $1-(N_{min}/N)^n$, because N is almost equal to N_{min} and thus value of N_{min}/N is almost one. Also the growth rate at the stationary phase is suppressed by the term of $1-(N/N_{max})^m$, because N is almost equal to N_{max} and thus value of N/N_{max} is almost one. The growth rate in the log phase is the maximum and almost constant. These characteristics of the model make it depict a sigmoidal growth curve. The model describes a sigmoidal curve on a semi-log plot, as shown below. Also it can describe a sigmoidal curve on the Cartesian plane (Fig. 2). This means that the model can be applied to describe the sigmoidal population curves of humans and other living things as well.

Parameter m is a curvature parameter. With a larger m, the curvature of the deceleration phase with the model is smaller (Fig. 3). Parameter n is a parameter related to the period of lag, *lag*. With a larger n, the period of lag is shorter (Fig. 4).

Growth at constant temperatures.

The new logistic model was compared with the modified Gompertz and Baranyi models for *E. coli* growth in nutrient broth at constant temperatures. All the models described growth curves well (Fig. 5) (Fujikawa et al. 2004). Curves described by the new logistic model were very similar to those by the Baranyi model. The Gompertz curves were more variable throughout growth.

For r and *lag*, which are the key parameters to characterize a growth curve, the model gave the best

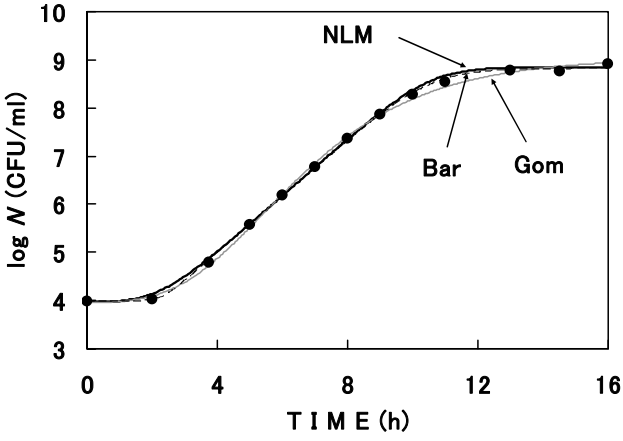

FIG. 5. Comparison of models for *E. coli* growth in broth at 27.6℃. Closed circles are measured values. NLM : New logistic model, Bar : the Baranyi model, Gom : the modified Gompertz model.

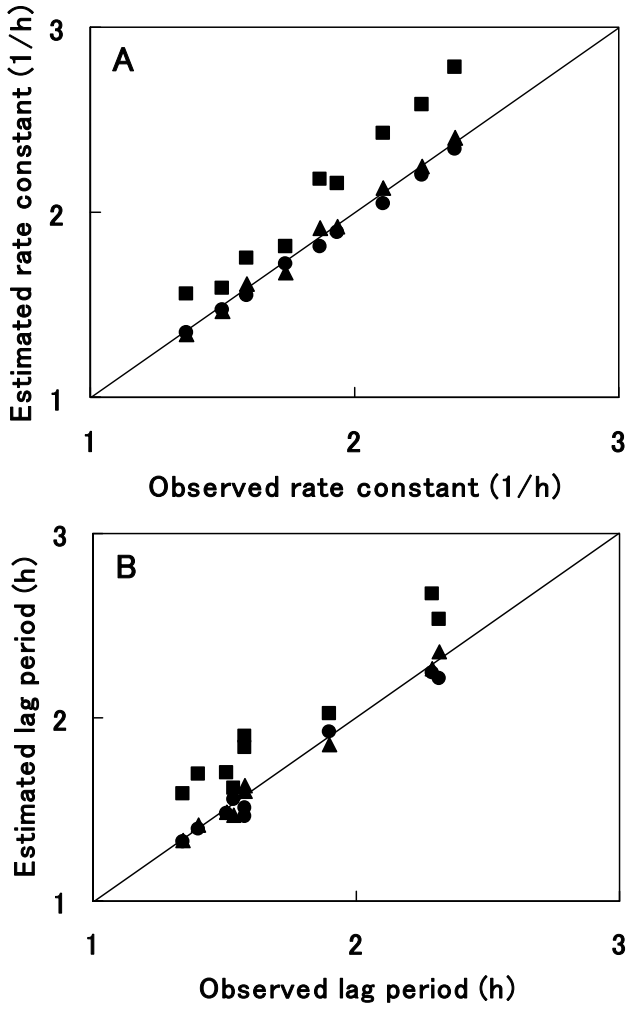

FIG. 6. Comparison of models for *E. coli* growth in broth: A. rate constant of growth, B. duration of the lag period. ●, New logistic model ; ▲, the Baranyi model ; ■, the modified Gompertz model. The straight line is the line of equivalence.

estimates among the models (Fig. 6A, B) (Fujikawa et al., 2004). These results were also obtained for *E. coli* growth on the surface of a nutrient agar plate (Fujikawa and Morozumi, 2005). The Gompertz model had a tendency to overestimate r and *lag* (Fig. 6A, B).

Temperature dependency of r for *E. coli* growth in broth was well described with the Arrhenius model (Fig. 7) (Fujikawa et al. 2004). The regression line in the figure was expressed as follows.

$$\ln r = 21.0 - 6230/T \qquad (3)$$

With this equation, we could estimate value of r at temperature T. Values of r at the temperatures studied were also precisely described with the square root model (Eq. 4) (McMeekin et al., 1993).

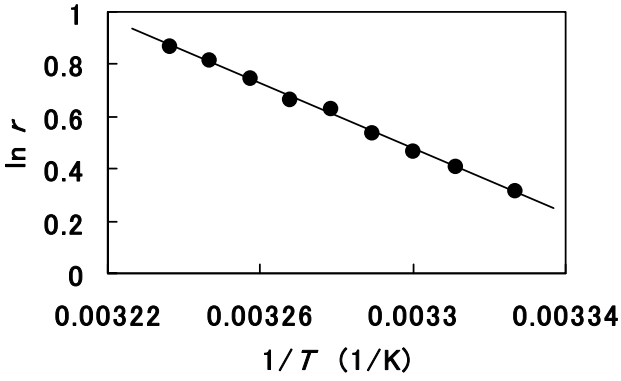

FIG. 7. Temperature dependency of the rate constant for *E. coli* growth in broth. Closed circles are measured values. The straight line depicts the linear regression line.

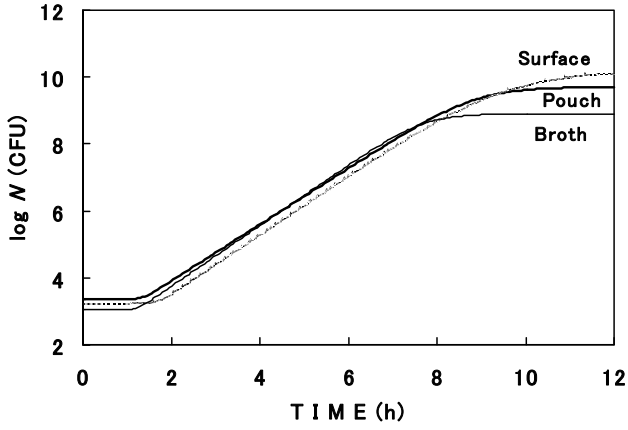

FIG. 8. *E. coli* growth curves at 34℃ under various conditions. Curves show growth in broth, on agar plate surface, and in a pouched food that is described with the new logistic model. The unit of the viable cell number is expressed as CFU only, because each unit varies with its environment, like CFU/g and CFU/ml.

$$\sqrt{r} = 0.0452\ (T - 1.93) \qquad (4)$$

On the other hand, growth curves under various atmospheric conditions at a given temperature were very similar to each other. *E. coli* growth curve in nutrient broth was very similar to those on the surface of a nutrient agar plate and in mashed potatoes in a pouch (Fig. 8) (Fujikawa et al., 2006a). These results suggested that regardless of the differences in physical (atmospheric) conditions, the growth kinetics of bacterial cells (facultative microbes) were identical at places where the nutrient conditions were good. Furthermore, it means that a growth model can describe microbial growth at those places.

Growth prediction at dynamic temperatures.

The temperature of an actual food from its production to consumption varies with time. To predict microbial growth at dynamic temperatures with a mathematical model, the rate constant of growth, r at time t during the period is needed. Value of r is estimated from temperature T at time t with the Arrhenius model (Eq. 3) and then embedded into the growth model (Eq. 2). The new logistic model successfully predicted *E. coli* growth at dynamic temperatures under various conditions. That is, the model could precisely predict bacterial growth in broth (Fig. 9), on the surface of a nutrient agar plate (Fig. 10), and in mashed potatoes in a pouch (Fig. 11) (Fujikawa et al., 2004; Fujikawa and Morozumi, 2005; Fujikawa et al., 2006b). The prediction with the model was very similar to that with the Baranyi model, as shown in

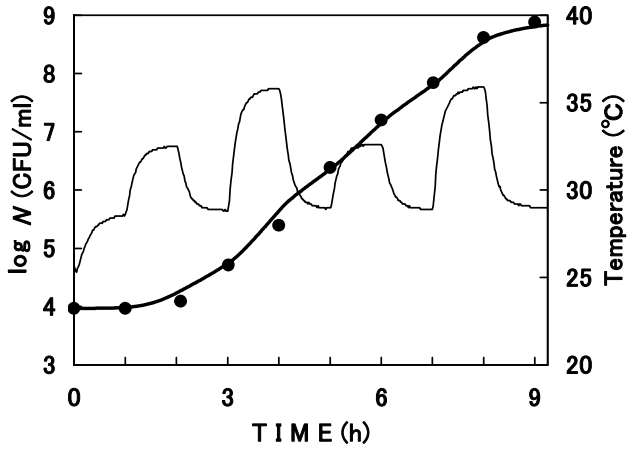

FIG. 9. *E. coli* growth prediction in broth with the new logistic model. Closed circles are measured cell numbers. Thick and thin lines depict the growth predicted with the model and the temperature of the broth, respectively.

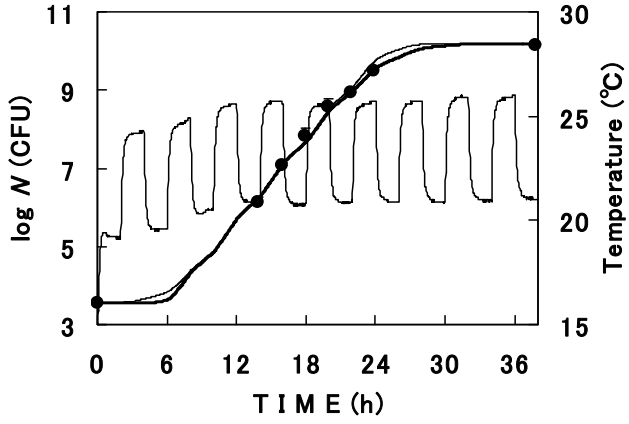

FIG. 10. *E. coli* growth prediction on a nutrient agar plate surface with the new logistic model. Closed circles are measured cell numbers. Thick and gray lines depict growth predicted with the new logistic model and the Baranyi model, respectively. A thin periodic line depicts the temperature of the plate.

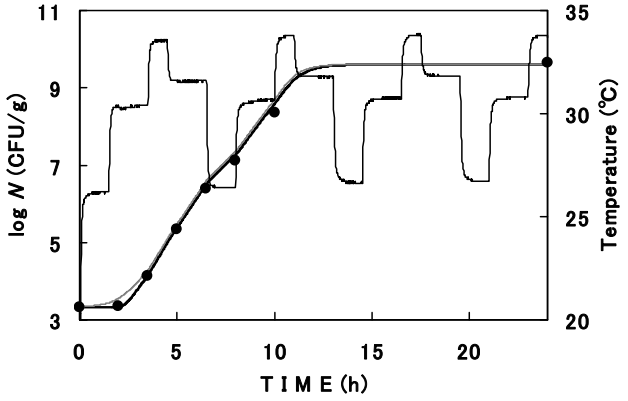

FIG. 11. *E. coli* growth prediction in a pouched food with the new logistic model. Closed circles are measured cell numbers. Thick and gray lines depict growths predicted with the new logistic model and the Baranyi model, respectively. A thin periodic line depicts the temperature of the plate.

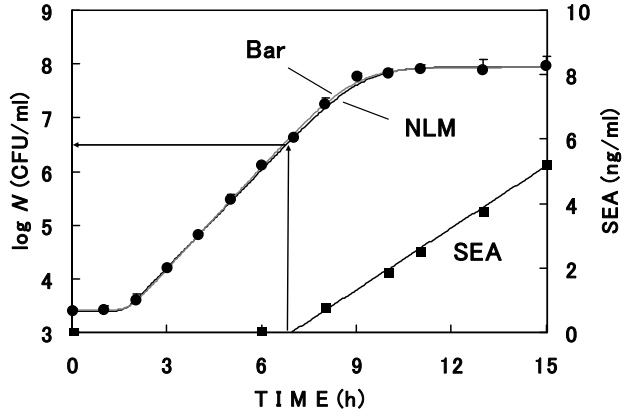

FIG. 12. *S. aureus* growth and SEA production in milk at 32℃. ●, measured cell numbers; ■, measured SEA amount. Thick and gray lines depict the growth predicted with the new logistic model and the Baranyi model, respectively. The straight line is the line of linear regression for the SEA amount. The arrows show the intersection of the regression line of SEA with the horizontal axis corresponding with a point at the staphylococcal cell number of about $10^{6.5}$ CFU/ml.

Fig. 10 and 11.

These results showed that the new logistic model could precisely describe and predict microbial growth at various patterns of temperature.

Prediction of microbial toxin production.

Staphylococcal enterotoxins are harmful metabolites that cause emesis in humans. There was an widespread *Staphylococcus aureus* food poisoning outbreak among patients who ingested dairy products in Osaka, Japan in 2000. Thus, *S. aureus* growth and its enterotoxin production in commercial milk products were studied with the new logistic

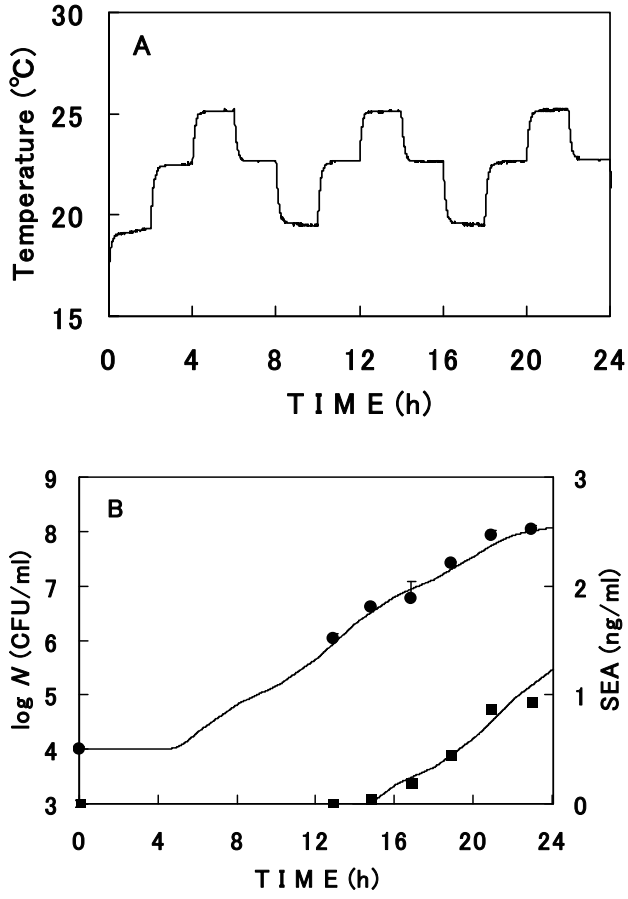

FIG. 13. Prediction of *S. aureus* growth and SEA production in milk at a dynamic temperatures with the new logistic model. A. the temperature profile of the milk. B. ●, measured cell numbers; ■, measured SEA amount. Curves are described with the model.

model. *S. aureus* growth in milk at constant temperatures from 14 to 36.5℃ was precisely described with the model, similar to the *E. coli* growth studied above. The amount of staphylococcal enterotoxin A, SEA in milk initially increased linearly with time from a time when the cell population reached about $10^{6.5}$ CFU/ml at these temperatures (Fig.12) (Fujikawa and Morozumi, 2006). Thus, the toxin production was supposed to be a zero-order reaction. The rate constant of the reaction was also evaluated at various temperatures. With parameter values obtained at the constant temperatures, the model successfully predicted bacterial growth in milk at varying temperatures. For the toxin level estimation, we postulated that the rate of toxin production might be regulated with the temperature after the cell concentration reached $10^{6.5}$ CFU/ml; the time point when the cell concentration reached that value was predicted with the growth model. The toxin production predicted with this algorithm was about two times greater than

that measured. With a correction factor in the toxin estimation, the toxin level in milk was successfully predicted at varying temperatures (Fig. 13) (Fujikawa and Morozumi, 2006). These results showed that this prediction system consisting of the growth model and the toxin production algorithm might be a useful tool for modeling the growth of bacteria and their metabolite production in foods.

The new logistic model has been found to be a useful tool to analyze and predict microbial growth in food, as reviewed above. In the near future we would like to show how the model can be applied to actual food safety systems.

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