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Note

Prediction of the Growth of *Salmonella* Enteritidis in Raw Ground Beef at Various Combinations of the Initial Concentration of the Pathogen and Temperature

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Recently we clarified the growth kinetics of *Salmonella* Enteritidis in raw ground beef at various temperatures with our growth model. Based on those results, this study aimed to build a new methodology to predict the growth of *Salmonella* in ground beef at given initial concentrations of the pathogen and temperatures. Namely, the maximum cell population of *Salmonella* at various combinations of its initial concentration and temperature was developed with a polynomial equation. The rate constants of *Salmonella* growth at various temperatures were estimated with the square root model studied in our recent study. A new system consisting of our growth model, the polynomial equation, and the square root model successfully predicted the growth of *Salmonella* inoculated at given concentrations in beef at constant and dynamic temperatures. The growth of natural microflora in beef at those temperature patterns were also successfully predicted with the growth model.

Key words：*Salmonella* / Ground beef / Growth prediction / Logistic model / Polynomial model.

Salmonella is still one of the major pathogens that cause food-borne diseases to humans. Especially, *Salmonella* Enteritidis has been ranked at the top of fifteen most common *Salmonella* serovars isolated from people in 37 countries from 2001 to 2007 and between 2010 and 2014 in Japan (Hendriksen et al., 2011; IDSC, 2014). *Salmonella* Enteritidis was also one of the most common serotypes causing beef-associated outbreaks (accounting for 18%) in the USA between1998 and 2008 (Jackson et al., 2013).

An effective strategy to control food-borne salmonellosis needs to be made with the knowledge of the growth kinetics of the pathogen in ground beef. However, few studies have been published on the growth kinetics of *Salmonella* in ground beef (Mackey and Kerridge, 1988; Juneja et al., 2009). The growth characteristics of the pathogen in ground beef have not been fully clarified.

The authors have so far studied *Salmonella* Enteritidis growth in raw ground chicken and liquid egg products at dynamic temperatures with a growth model so far (Zaher and Fujikawa, 2011; Sakha and Fujikawa, 2012 and 2013). Recently the authors (Sabike et al., 2015) clarified and predicted the growth of *Salmonella* Enteritidis in ground beef at dynamic temperatures with the growth model. The prediction at the dynamic temperatures was successful, but it was at a single initial concentration of the pathogen. Also, the growth of the pathogen at various initial concentrations was studied, but it was at a constant temperature only (Sabike et al., 2015). The growth of the pathogen at various combinations of initial concentration and temperature was not studied in that investigation.

There seem to be so far no papers on models that have successfully predicted the growth of a pathogen from various initial concentrations and temperatures in raw food. Koseki et al. (2011) studied the growth of

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Listeria monocytogenes in raw tuna with a mathematical model. In their study, the value for the maximum cell population, N_{max} for *L. monocytogenes* was modeled at different initial levels of natural microflora (NM) in tuna and the initial concentration of the pathogen was constant (2 log CFU/g). Therefore, in the present study, we tried to develop a model system that could predict the growth of *Salmonella* in ground beef at a given initial concentration, *I*, of the pathogen and temperature, *T*, for microbial food safety management.

Our recent study on the growth of *S.* Enteritidis in ground beef (Sabike et al., 2015) led to the following assumptions: (i) the value for N_{max} of the pathogen in ground beef might be determined with *I* and *T*, using a polynomial equation with *I* and *T* and (ii) the value for the rate constant, *r*, at any value of *I* might be determined only with *T*, using like the square root model. The growth model system with the above assumptions was studied for its applicability to the growth of the pathogen in ground beef in this study. Here it was thought that the values for other parameters in the growth model would be constant, as shown in the growth prediction in ground beef (Sabike et al., 2015) and in ground chicken and liquid egg products (Zaher and Fujikawa, 2011; Sakha and Fujikawa, 2012 and 2013).

Four *S.* Enteritidis strains SE2, SE3, SE5 and 04-137 were used for the study, which were the same strains in our recent study (Sabike et al., 2015). Briefly, cells of the strains activated on XLD agar plates were incubated in Trypticase soy broth. Cultured cells were washed and then thoroughly suspended in saline, yielding a cell suspension of about 10^9 CFU/ml. A cocktail of the four *Salmonella* suspensions with equal volumes was then made. The cell suspension was then diluted to various concentrations with saline.

The ground beef was the same as that used in our recent study (Sabike et al., 2015). Samples were then prepared in the same manner as were done previously (Zaher and Fujikawa, 2011; Sakha and Fujikawa, 2012 and 2013; Sabike et al., 2015). Briefly, ground beef samples were inoculated with the *Salmonella* cell suspension prepared above (2 ml/100 g beef). After thorough mixing, 10-g portions of it were placed in glass bottles. The bottles were then stored at a constant temperature. Immediately after incubation, each sample (one bottle per data point) was taken from the incubator. Three trials were performed at each constant temperature. For the dynamic temperature experiment, the bottles were placed in a programmable incubator. The temperature of the sample was monitored throughout the experiment with a digital thermometer. Immediately after each incubation period, the sample in triplicate was taken from the incubator.

Bacterial cell counts in the ground beef samples were done in the same manner as in our previous studies (Zaher and Fujikawa, 2011; Sakha and Fujikawa, 2012 and 2013, Sabike et al., 2015). Briefly, the beef samples in the bottles were mixed with buffered sodium chloride peptone solution to make 10% food homogenates. The sample homogenate was serially 10-fold diluted with saline (Anonymous, 2004). Total (aerobic) bacteria counts of the sample were enumerated in duplicate with the surface-plating method using standard method agar plates (Anonymous, 2004). *Salmonella* counts of the sample were enumerated in duplicate with the surface-plating method using XLD agar plates. The counts of NM were calculated by subtracting the *Salmonella* counts from the total bacteria counts for each sample (Sabike et al., 2015). The average count with two plates was then obtained for NM and *Salmonella* for each data point.

Average counts of *Salmonella* and NM were then calculated for the samples at the constant or dynamic temperature (Sabike et al., 2015). These counts of *Salmonella* and NM during the storage were then analyzed with the extended logistic model, which is expressed as follows (Fujikawa et al., 2003; Fujikawa and Morozumi, 2005):

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

Here *N* is the population of a microorganism (CFU/g) at time *t* (h), *r* is the rate constant of growth (1/h), N_{max} is the maximum population (CFU/g), and N_{min} is the initial population (CFU/g). *m* and *n* (>0) are parameters related to the curvature of the deceleration phase and the period of the lag phase, respectively. Numerical data of microbial counts were analyzed by a computer program to fit the data to the growth model (Fujikawa and Kano, 2009).

The value for N_{max} of *Salmonella* at a given combination of *I* and *T* was estimated with a polynomial equation of the third order. The parameters in the polynomial equation were obtained with the Solver function in Microsoft Excel.

The performance of a mathematical model or an equation was evaluated with the square root of the mean of the square error, *RMSE*, between log-transformed cell concentrations estimated with the model (log N_{est}) and those observed (log N_{obs}) at the observation points (Sabike et al., 2015). Regression analysis was performed with Microsoft Excel.

The values for N_{max} of the pathogen in ground beef at various combinations of *I* and *T* were first studied in the present study. The examined values for *I* and *T* were 2, 3, and 4 log CFU/g and 16, 20, 24, and 28℃,

respectively. Thus, there was a total of 12 (4×3) growth curves and the values of N_{max} were obtained from the stationary phases of the corresponding growth curves. A cubic equation for N_{max} was then developed to fit the values at these combinations of *I* and *T*, as shown in equation 2.

$$N_{\max} = 1.3\times10^{-6}T^3 + 4.2\times10^{-4}I^3 - 3.9\times10^{-7}T^2I + 1.8\times10^{-5}TI^2 + 2.7\times10^{-3}T^2 + 9.2\times10^{-4}TI + 8.3\times10^{-4}I^2 + 6.5\times10^{-4}T + 5.7\times10^{-1}I + 4.7 \quad (2)$$

Here the values for these terms in the equation were estimated by minimizing the *RMSE* value for N_{max}. The *RMSE* value for this equation was very low at 0.14 log CFU/g. Figure 1 graphically shows the N_{max} values estimated at given values for *T* and *I* using equation 2. As shown in the figure, the estimated value for N_{max} was generally higher at higher values for *T* and *I*. With equation 2, the value for N_{max} at a combination of *T* and *I* could be estimated in the ranges of 16-28℃ for *T* and 2-4 log CFU/g for *I*.

A prediction system at various values for *T* and *I* was then developed using the primary growth model. Namely, the value for N_{max} in the growth model (equation 1) was estimated by equation 2. The value for *r* in equation 1 was estimated with the square root model (equation 3), which was obtained from our recent study (Sabike et al., 2015).

$$\sqrt{r} = 0.0401(T - 3.47) \quad (3)$$

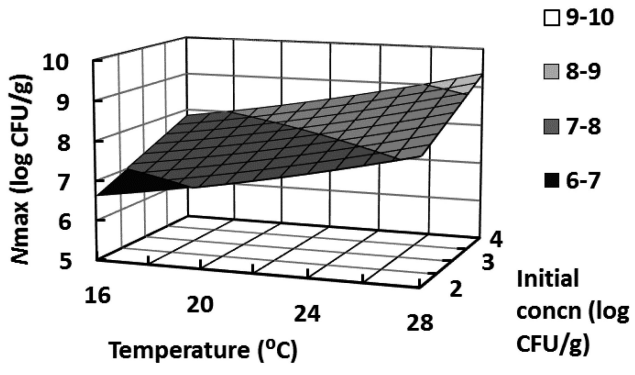

FIG. 1. Three dimensional plot of the maximum population for *Salmonella* estimated with the polynomial equation at given values for the initial concentration of the pathogen and temperature.
The plot is divided into four sections with the estimated values.

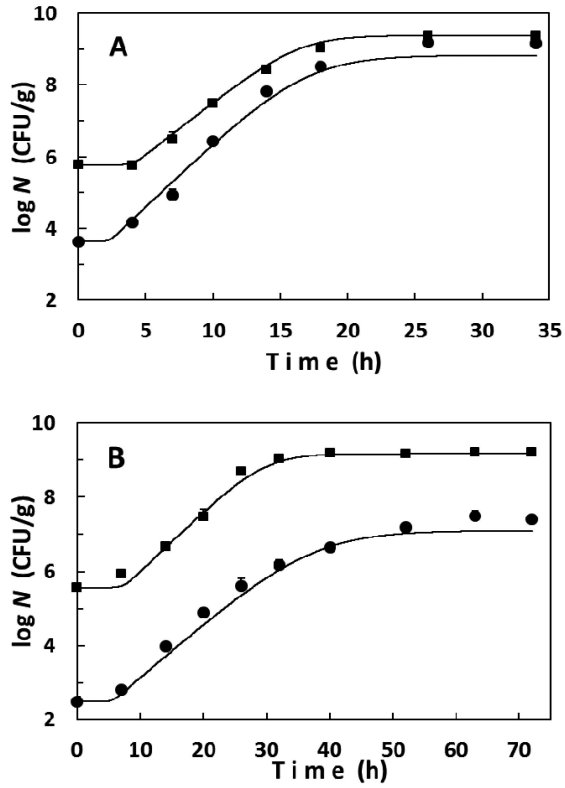

FIG. 2. Prediction of the growth of *Salmonella* and NM at given values for the initial concentration of the pathogen and constant temperatures at 26℃ (A) and 18℃ (B).
Symbols:●, *Salmonella*;■, NM. Bars which show the standard deviations at data points are too small to appear for most points. Curves are depicted with the growth model system.

The average values for *m* (0.47) and *n* (6.4) obtained in our recent study (Sabike et al., 2015) were used in equation 1 for prediction.

The above system was examined for the prediction of the growth of *Salmonella* at constant temperatures. The system successfully predicted the growth of the pathogen in ground beef at A. 26 and B. 18℃ (Fig. 2). Here the values for *I* were A. 3.6 and B. 2.5 (log CFU/g). The *RMSE* values in Fig. 2 for *Salmonella* were low at A. 0.27 and B. 0.25 (log CFU/g).

The growth of NM in ground beef was predicted with equation 1 in the same manner as in our previous papers (Zaher and Fujikawa, 2011; Sakha and Fujikawa, 2012 and 2013). Parameters in the equation were those obtained in our recent study (Sabike et al., 2015). The growth of NM was also successfully predicted with the model (Fig. 2). The *RMSE* values for NM were also very low, which are A. 0.097 and B. 0.15 (log CFU/g).

The system was then evaluated at dynamic temperatures. It also precisely predicted the growth of

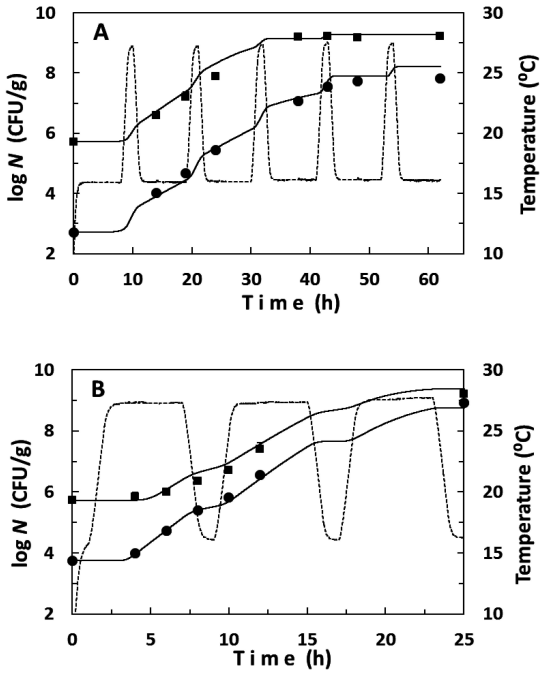

FIG. 3. Prediction of the growth of *Salmonella* and NM at given values for the initial concentration of the pathogen and at dynamic temperatures.
Symbols:●, *Salmonella*;■, NM. Bars which show the standard deviations at data points are too small to appear for most points. Curves are depicted with the growth model system. Dotted lines show measured temperatures of ground beef.

the pathogen at dynamic temperatures (Fig. 3); the *RMSE* values in the figures for *Salmonella* were low at A. 0.20 and B. 0.10 (log CFU/g). Here the values for I were A. 2.7 and B. 3.7 (log CFU/g).

The growth of NM at the dynamic temperatures was also successfully predicted from the temperature history (Fig. 3). The *RMSE* values for NM were also very low, which are A. 0.19 and B. 0.19 (log CFU/g).

A very high linearity was found between the values predicted and measured for *Salmonella* in Figs. 2 and 3 (Fig. 4). The total of the points were 29 in the figures. Most points were located on or close to the line of equivalence. The linear regression line for all the points in the figure was expressed as

$$Y = 0.985\,X \qquad (4)$$

where X and Y are the measured and predicted populations (log CFU/g), respectively. Here the intercept of the regression line was set to be zero, because the line needed to start with the origin in the plot. The value for the slope (0.985) was very close to

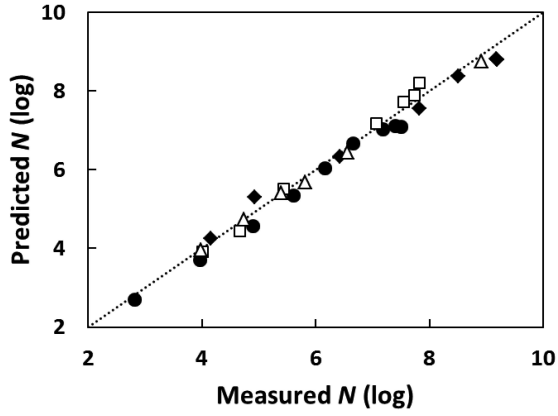

FIG. 4. Linearity between predicted and measured populations for *Salmonella* in ground beef.
All data points in Figs. 2 and 3 were plotted. The straight line is the line of equivalence.
Symbols; ◆, points in Fig. 2A; ●, those in Fig. 2B; □, those in Fig. 3A; △, those in Fig. 3B.

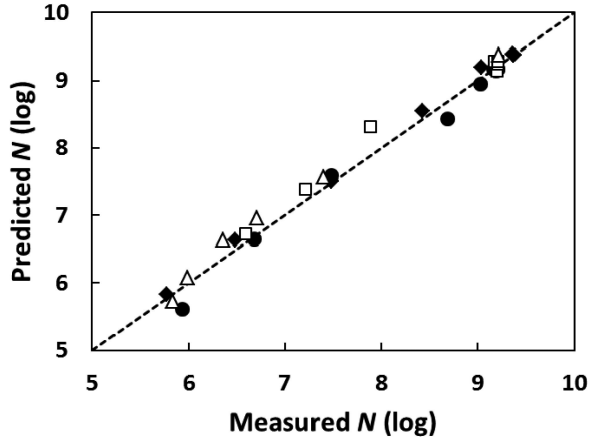

FIG. 5. Linearity between predicted and measured populations for NM in ground beef.
All data points in Figs. 2 and 3 were plotted. The straight line is the line of equivalence.
Symbols; ◆, points in Fig. 2A; ●, those in Fig. 2B; □, those in Fig. 3A; △, those in Fig. 3B.

one, indicating that the predicted *Salmonella* populations were almost equal to the measured ones. The coefficient of linearity for all the points in Fig. 4 was as high as 0.993. The coefficients of linearity at the constant and dynamic temperatures were both very high, being 0.995 and 0.996, respectively.

A very high linearity between predicted and measured populations was also found for NM in Figs. 2 and 3 (Fig. 5). The linear regression line for all the points in the figure was expressed as follows.

$$Y = 1.01X \quad (5)$$

The value for the slope (1.01) was also almost equal to one. The coefficient of linearity for all the points in Fig. 5 was also as high as 0.993.

The residual which is the value of the measured population minus the predicted one (log CFU/g) (Oscar, 2009) was also very small for *Salmonella*; all residuals for the data points in Figs. 2 and 3 were located between 0.5 and -0.5 (log), showing that all points were in the acceptable prediction zone between -1 log and 0.5 log (Oscar, 2009). The average of the residual for *Salmonella* was also small (0.091 log). The residuals may vary with the experimental designs, the models used, and so on, but the residuals obtained in this study were smaller than those for *L. monocytogenes* in tuna (Koseki et al., 2011) or *Salmonella* on chicken skin (Oscar, 2009).

Similar results were obtained in the residuals for NM in ground beef. Namely, all residuals for NM in Figs. 2 and 3 were also very small and between 0.5 and -0.5 (log). The average for all points was as small as -0.054 (log).

To our knowledge, the present study would be the first report on the successful prediction of the growth curve for a microbe of concern in raw food at a given initial microbial concentration of the microbe and a variety of temperatures. Oscar (2009) studied the growth of *Salmonella* on chicken skin based on the inoculum size with a general regression neural network model, but the author reported that the model had local prediction problems at several combinations of the initial level of the pathogen and temperature. Also the growth was modeled at constant temperatures only (Oscar, 2011).

The cubic polynomial equation (equation 2) was applied for the precise prediction of the N_{max} value in this study. A model with a quadratic equation, which is shown below, was preliminarily studied for the estimation for N_{max} for *Salmonella*.

$$N_{\max} = 5\times10^{-5}T^2 + 6.4\times10^{-3}I^2 + 2\times10^{-5}TI + 1.1\times10^{-1}T + 5.4\times10^{-1}I + 3.8 \quad (6)$$

The prediction with equation 6 for *Salmonella* incubated at the constant and dynamic temperatures was also good, similar to the cubic equation (data not shown). However, the prediction at dynamic temperatures in Fig. 3B was not as good with the *RMSE* value of 0.13, whereas the value generated by the cubic equation was smaller (0.10), as described above. Thus, the cubic equation was adapted as the estimation model in the present study.

In the present study, we could develop a model system for *Salmonella* growth in ground chicken at various combinations of the initial concentration of the pathogen and temperature. However, the range of the initial concentration and temperature studied here might not be wide enough to cover the actual storage and transportation conditions of ground beef. Thus, a further study using a wider range of the initial concentration and temperature will be needed in the future. Also, it should be examined whether the present system could be applied to the growth of other microbes in raw foods.

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REFERENCES

Anonymous (2004) Standard method of analysis in food safety regulation. Japan Food Hygiene Association, Tokyo, Japan.

Fujikawa, H., Kai, A., and Morozumi, S. (2003) A new logistic model for bacterial growth. J. *Food Hyg. Soc. Japan*, **44**, 155-160.

Fujikawa, H., and Morozumi, S. (2005) Modeling surface growth of *Escherichia coli* on agar plates. *Appl. Environ. Microbiol*., **71**, 7920-7926.

Fujikawa, H., and Kano, Y. (2009) Development of a program to fit data to a new logistic model for microbial growth. *Biocontrol Sci*., **14**, 83-86.

Hendriksen, R.S., Vieira, A.R., Karlsmose, S., Danilo, M.A., Wong, L.F., Jensen, A.B., Wegener, H.C., and Aarestrup, F.M. (2011) Global Monitoring of *Salmonella* Serovar distribution from the World Health Organization Global Foodborne Infections Network Country Data Bank: Results of Quality Assured Laboratories from 2001 to 2007. *Foodborne Pathog. Dis*., **8**, 887-900.

IDSC (Infectious Disease Surveillance center) (2014) Fifteen most common *Salmonella* serovars from human sources. (Prefectural/municipal public health institutes and health centers) Data Based on the reports received before February 4, 2014. http://idsc.nih.go.jp/iasr/virus/graph/salm0509.pdf.

Jackson, B.R., Griffin, P.M., Cole, D., Walsh, K., and Chai, S.J. (2013) Outbreak-associated *Salmonella enterica* serotypes and food commodities, United States, 1998–2008. *Emerg. Infect. Dis*., [serial online] **19**. http://dx.doi.org/10.3201/eid1908.121511.

Juneja, V.K., Melendres, M.V., Huang, L., Subbiah, J., and Thippareddi, H. (2009) Mathematical modeling of growth of *Salmonella* in raw ground beef under isothermal conditions from 10 to 45℃. *Int. J. Food Microbiol*., **131**, 106–111.

Koseki, S., Takizara, Y., Miya, S., Takahashi, H., and Kimura, B. (2011) Modeling and predicting the simultaneous growth of *Listeria monocytogenes* and natural flora in

minced tuna. *J. Food Prot*., **74**, 176-187.
Mackey, B.M., and Kerridge, A.L. (1988) The effect of incubation temperature and inoculum size on growth of *salmonellae* in minced beef. *Int. J. Food Microbiol*., **6**, 57-65.
Oscar, T.P. (2009) General regression neural network and Monte Carlo simulation model for survival and growth of *Salmonella* on raw chicken skin as a function of serotype, temperature, and time for use in risk assessment. *J. Food Prot*., **72**, 2078-2087.
Oscar, T. P. (2011) Extrapolation of a predictive model for growth of a low inoculum size of *Salmonella* Typhimurium DT104 on chicken skin to higher inoculum sizes. *J. Food Prot*., **74**, 1630-1638.
Sabike, I.I., Fujikawa, H., and Edris, A.M. (2015) The growth kinetics of *Salmonella* Enteritidis in raw ground beef. *Biocont. Sci*., **20**, 183-190.
Sakha, M. Z., and Fujikawa, H. (2012) Growth characteristics of *Salmonella* Enteritidis in pasteurized and unpasteurized liquid egg products. *Biocontrol Sci*., **17**, 183-190.
Sakha, M. Z., and Fujikawa, H. (2013) Prediction of *Salmonella* Enteritidis growth in pasteurized and unpasteurized liquid egg products with a growth model. *Biocontrol Sci*., **18**, 89-93.
Zaher, S.M., and Fujikawa, H. (2011) Effect of native microflora on the growth kinetics of *Salmonella* Enteritidis strain 04-137 in raw ground chicken. *J. Food Prot*., **74**, 735-742.