**Mathematical Modeling and Validation of Growth of *Salmonella* Enteritidis and
Background Microorganisms in Potato Salad – One-Step Kinetic Analysis and Model
Development[†]**

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Running head: Growth of *Salmonella* Enteritidis in potato salad

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Abstract

This study was conducted to examine the growth of *Salmonella* Enteritidis (SE) in potato salad caused by cross-contamination and temperature abuse, and develop mathematical models to predict its growth. The growth of SE was investigated under constant temperature conditions (8, 10, 15, 20, 25, 30, and 37°C) to evaluate the effect of temperature on growth rates and lag times. Duplicated experiments were conducted. The data set from one replicate was used to develop kinetic models and determine kinetic parameters. The data from the other replicate served as an independent data set for model validation. The growth of background microorganism (BK) was also examined.

One step-kinetic analysis method was used to directly construct both primary (Huang) and secondary (Ratkowsky square-root) models. Nonlinear regression was used to minimize the global residual sum of squares (RSS) for SE and BK. The results showed that both primary and secondary models can be used to analyze the growth curves, with the kinetic parameters closely matching the characteristics of SE and BK. The validation results showed that the root-mean-square error (RMSE) was only 0.40 Log CFU/g for SE and 0.66 Log CFU/g for BK, with the residual errors of predictions following Laplace and logistic distributions, respectively.

This study showed that one-step kinetic analysis is a useful and efficient method for analyzing the entire data set to directly construct primary and secondary growth models and determine kinetic parameters. Since the models are validated, they can be used to predict the growth of SE and conduct risk assessment, and to predict the microbiological shelf-life of potato salad.

Keywords: *Salmonella* Enteritidis, modeling, potato salad, one-step kinetic analysis

1. Introduction

According to a study reported by the Centers for Diseases Control and Prevention (CDC), non-typhoidal *Salmonella* is responsible for approximately 1.2 million cases of foodborne infections and 450 deaths each year in the United States (Scallan et al. 2011). Among more than 2,500 different serotypes, *Salmonella* serotype Enteritidis (SE), *Salmonella* serotype Typhimurium, and *Salmonella* serotype Newport are three of the most common serotypes causing foodborne infections in the United States (CDC, 2014). Among laboratory-confirmed cases of *Salmonella* infections caused by top 20 serotypes, SE appears to be the top frequently reported serotype (CDC, 2013, 2014). Most SE infections in the United State are acquired from domestic sources (Chai et al., 2012). Chicken and eggs are no doubt the major sources and vehicles for the transmission of SE (Braden, 2006; Chai et al., 2012; Guard-Petter, 2001).

Chicken eggs are widely used as an ingredient in making a variety of salads, both at home and in commercial settings. Cross-contamination can occur when processing surfaces, kitchen utensils, and cutting boards contaminated with SE are in contact with various materials used to make salads. Therefore, opportunities exist that salads may be contaminated with SE through contaminated ingredients or contact surfaces. Once contaminated, salads may become a health risk to consumers. A study reported by Alali et al. (2012) showed that *Salmonella* survived in highest numbers in potato salad and hummus. Between 1988 and 1992, at least one *Salmonella* outbreak was related to potato salad (CDC, 1996). In 2005, cross-contamination of SE between eggs and potato caused salmonellosis in 85 out of 115 people attending a local traditional fair in an Austrian village after eating mixed salad containing potato (Schmid et al., 2006). Potato salad was also identified as the source in another SE outbreak in Austria in 2011

(Hrivniaková et al. 2011). In 2010, Safeway Inc. voluntarily recalled potato salad from 10 states over the concern of *Salmonella* contamination.

The objective of this research was to study the growth kinetics of SE in potato salad exposed to cross-contamination during preparation, and to evaluate the effect of temperature abuse on growth rate and lag time under different storage temperatures. The growth of background microorganisms (BK) in potato salad was also evaluated. The goal of this research was to develop mathematical models to describe the growth of SE and BK in potato salad. The models may be used for conducting risk assessments of SE in potato salad and determining microbial shelf-life during storage.

2. Materials and Methods

2.1 SE strains and preparation of bacterial cultures

Five outbreak SE strains, obtained from the culture collection of the Eastern Regional Research Center (ERRC) of the USDA Agricultural Research Service (ARS) located in Wyndmoor, PA, were used in this study. The SE strains included FSIS OB030832, FSIS OB040159, FSIS OB050042, FSIS OB060003, and SE Phage Type (PT) 8 strain C405. The working cultures of the bacterial strains were prepared by inoculating each individual strain to brain heart infusion (BHI) broth (BD/Difco Laboratories, Sparks, MD) and incubated at 37°C overnight with mild agitation (~100 rpm). Each strain obtained from the working culture was induced to resist 100 mg/L rifampicin (rif, Sigma, R 3501-5G, Sigma Aldrich Co., MO) using the procedures described in Fang et al. (2013). After the antibiotic resistance was consistently induced to each strain, the bacterial strains resistant to rif (SE-rif) were used in all growth studies.

The advantage of using SE-rif in growth studies was that it simplified the microbiological procedures during isolation and enumeration of SE from the samples that contained significant amount of background microorganisms by suppressing their growth. To maintain the viability of the cells and stability of the antibiotic resistance, the SE-rif cultures were plated onto Tryptic Soy agar (TSA, BD/Difco Laboratories) plates containing 100 mg/L rif (TSA-Rif) and stored in a refrigerator (6-8°C). The SE-rif cultures were regularly propagated, maintained on TSA-rif agar plates, and refrigerated. The growth behaviors of SE were not affected by the induction of the rif-resistance in preliminary experiments.

To prepare the bacterial cultures for inoculation, a loopful of each SE-rif strain was transferred to 10 ml BHI broth containing 100 mg/L rif and incubated overnight (22-24 h) at 37°C with mild shaking (~100 rpm). The overnight cultures were harvested by centrifugation (2400 g, 15 min, and 4°C), washed once with 0.1% sterile peptone water (PW, BD/Difco Laboratories). Each bacterial culture was re-centrifuged, re-suspended in 5 ml PW, and then combined to form a 25 ml bacterial cocktail, which contained $\sim 10^9$ colony forming units (CFU) per ml. The bacterial cocktail was properly diluted for use in inoculation of samples. Fresh SE-rif cultures were prepared for each growth study.

2.2 Sample preparation, inoculation, and incubation

Frozen potato cubes (Southern Style Hash Browns) were purchased from a local grocery store. The potato cubes were kept in a freezer and remained frozen ($\sim -18^\circ\text{C}$) until ready for use in growth studies. To prepare for samples for use in the growth studies, the frozen potato cubes (~ 250 g) were placed into a plastic bag, and cooked in a microwave oven (1300 W) for 2 min on

high power, according to the manufacturer's suggestion. After microwave cooking, the samples were cooled in a refrigerator ($\sim 4^{\circ}\text{C}$) for at least 30 min.

After cooling, the potato samples were transferred to a sterile beaker containing 297 ml PW. The SE-rif culture (3 ml) was inoculated to the beaker and gently stirred for 2 min to allow uniform distribution of the bacteria. The liquid portion was drained after mixing. The inoculated potato cubes (125 g) were mixed with 33 g Hellmann's Real Mayonnaise (Unilever US, Inc., Englewood Cliffs, NJ). According to the manufacturer's label, the ingredients in the mayonnaise included soybean oil, water, whole eggs, egg yolks, vinegar, salt, sugar, lemon juice, EDTA, and natural flavors. The pH of the mayonnaise was measured as 3.7 using a Corning pH meter (Model 430, Corning Inc., New York). This value was identical to the value reported in Hwang and Tamplin (2005), in which Hellmann's Real Mayonnaise was also used. The mayonnaise contained 90 mg of salt per serving (13 g), or which is equivalent to 0.7% of salt in the product. The water activity of the mayonnaise was 0.95 (Hwang and Tamplin, 2005). After mixing, the potato salad (10g) was weighed into filter stomacher bags (Whirl-Pak[®], 7 oz., 95 mm $\times$ 180 mm $\times$ 0.08 mm, NASCO - Fort Atkinson, Fort Atkinson, WI). The inoculated samples of potato salad were kept in a refrigerator ($\sim 4^{\circ}\text{C}$) overnight and used for growth studies the next day.

2.3 Growth studies

The second day after sample inoculation, the inoculated samples were retrieved from the refrigerator and immediately transferred to incubators maintained at constant temperatures under suboptimal conditions between 8 and 37°C . The incubation temperatures in this study included 8,

10, 15, 20, 25, 30, and 37°C. A thermocouple was inserted to the center of a sample package to monitor the change in the sample temperature during incubation in each incubator. The temperature probe was also used to determine the time that the samples needed to increase from the refrigerated temperature to the incubation temperature in each incubator. This time is normally known as the come-up time (CUT) in heat transfer. The CUT was generally less than 30 min. For incubation temperatures below 15°C, the CUT was negligible and ignored since the incubation time was very long. For incubation temperatures above 15°C, the incubation time was adjusted from the initial CUT to allow the samples to equilibrate to the incubation temperature in each incubator. The samples were incubated for different durations of time, depending on the incubation temperature, and periodically retrieved to enumerate SE and BK. The experiments were duplicated for each growth temperature.

2.4 Enumeration of microorganisms

After the samples were retrieved from the incubators, PW (10 ml) was added to each sample bag. The samples were pulsed for 2 min on each side at the maximum speed in a stomacher (Model BagMixer® 100W, Interscience Co., France). A small volume of the liquid portion of the stomached samples was plated, directly or after serial dilution, onto TSA-rif agar plates. The plates were incubated at 37°C overnight. Since the TSA agar plates were supplemented with rif, all background microorganisms were suppressed. Only SE-rif grew into colonies on TSA-rif agar plates. The colonies were counted and converted to logarithms (base 10) of CFU/g. The samples were also plated onto TSA plates without rif to recover total aerobic microorganisms. The TSA plates were also incubated at 37°C overnight. The total plate

counts were determined from the colonies. The total colony counts were subtracted from the counts of SE-rif, and the difference was treated as the counts of background microorganisms (BK). The BK colony counts were also converted to the logarithms (base 10) of colony-forming units per g (CFU/g).

2.5 Mathematical Models and Data Analysis

2.5.1 Primary and secondary models

All growth curves observed in this study exhibited three phases, including lag, exponential, and stationary phases, except for a few growth curve of SE incubated at lower temperature conditions (8 and 10°C), in which the growth of SE was outcompeted by BK when the incubation time was longer than 220 hr. For growth curves with three phases, the growth kinetics can be described by a Huang primary model (Eq. (1), Huang, 2008, 2013).

$$Y(t) = Y_0 + Y_{max} - \ln\{e^{Y_0} + [e^{Y_{max}} - e^{Y_0}]e^{-\mu_{max}B(t)}\} \quad (1)$$

$$B(t) = t + \frac{1}{\alpha} \ln \frac{1+e^{-\alpha(t-\lambda)}}{1+e^{\alpha\lambda}} \quad (2)$$

For the primary model in Eqs. (1) and (2), the concentrations of microorganisms are expressed as the natural logarithms of bacterial counts, which include Y_0 , Y_{max} , and Y that represent the initial (time 0), maximum, and real time (t) concentrations of microorganisms. Included in Eqs. (1) and (2) are μ_{max} , the specific growth rate (h^{-1}), and λ , the lag time of a growth curve (h). In Eqs. (1) and (2), α is the lag phase transition coefficient, taking a value of 4

(Huang, 2013); and t is the incubation time under a constant temperature. The bacterial counts in logarithms of base 10 were converted to natural logarithms by multiplying 2.303.

Since all samples were incubated under suboptimal conditions, the effect of temperature on the specific growth rate of microbial growth was analyzed by the Ratkowsky square-root model (Eq. (3), Ratkowsky et al., 1982, 1983). The lag time (λ) in the Huang primary model is related to the specific growth rate by Eq. (4) (Fang et al. 2012; Huang, 2015a). In the secondary model for specific growth rates (Ratkowsky square-root model), a is the regression coefficient, T is the growth temperature, and T_0 is the nominal minimum growth temperature ($^{\circ}\text{C}$). In Eq. 4, both A and m are regression coefficients. Using this expression ensures a non-negative value of λ in the equation.

$$\sqrt{\mu_{max}} = a(T - T_0) \quad (3)$$

$$\ln(\lambda) = A - m \ln(\mu_{max}) \quad (4)$$

2.5.2 Data analysis and curve-fitting

Since duplicated experiments were performed in this study, the data were divided into two sets. One set of the data from one replicate was used for model development, and the other for model validation. The data analysis and curve-fitting was conducted using a one-step integrated approach reported by Huang (2015a, 2015b). Using this approach, all isothermal growth curves from one replicate were simultaneously analyzed to fit the primary model (Eq. (1)) and secondary models (Eqs. (3) and (4)) in one step, thus minimizing the global residual sum of

squares (RSS, Eq. (5)) for Y . In Eq. (5), N is the total number of data points in $\{Y\}$, and $\hat{Y}$ is the regression values, or the microbial counts (in natural logarithm) calculated from the models.

$$RSS = \sum_{n=1}^N (Y - \hat{Y})^2 \quad (5)$$

For one-step data analysis and curve-fitting, a SAS program was written to search for coefficients and parameters of the secondary models, initial inoculation level for each growth curve, and a global Y_{max} , labeled as $\{p\}$ (Eq. (6)). Because each temperature had a slightly different starting value Y_0 , the parameter vector $\{Y_0\}$ in Eq. 6 contained 7 parameters, one for each isothermal temperature. Therefore, the dimensions of the vector $\{p\}$ were 11×1 . The nlin procedure in SAS was programed using the Gauss-Newton method to search for optimal $\{p\}$ such that the RSS for the whole data set was minimized. For the SE and BK, the growth curves at 8°C were not used in curve-fitting, and were set aside for validation. For SE, the growth of bacteria was outcompeted by the background microorganisms when the incubation time was > 220 h at 10°C . Therefore, the data above 220 h were not used in curve-fitting for SE at 10°C . During preliminary data analysis, it was observed that the parameter A was not significantly different from zero. This parameter was set to zero in the subsequent analysis. Therefore, A is excluded from Eq. (6).

$$\{p\} = \begin{Bmatrix} a \\ T_0 \\ m \\ \{Y_0\} \\ Y_{max} \end{Bmatrix} \quad (6)$$

2.6 Model validation

One data set from the replicated experiments was used to validate the growth models obtained from this study. The distribution of residual errors ($\hat{Y} - Y$) were analyzed using @Risk (Version 7, Palisade Corp., Ithaca, NY). The root-mean-square error (RMSE) was calculated using Eq. (7), where q is the number of parameters.

$$RMSE = \sqrt{\frac{RSS}{N-q}} \quad (7)$$

3. Results and Discussion

3.1 One-step data analysis and model development

The initial concentration of SE inoculated to the samples was approximately 4 Log CFU/g, and the concentration of naturally existing BK was approximately 3.3 Log CFU/g. SE is a mesophilic bacterium, and grows well at warm temperature conditions. In this study, SE grew well at temperatures of 15, 20, 35, 30, 33, and 37°C, and developed into full growth curves under these temperature conditions. However, the growth of SE was outcompeted at low incubation temperatures (8 and 10°C) when the incubation time was greater than ~220 h, or after approximately 10 days of storage (Figs. 1 and 2). The background microorganisms in the samples may contain both mesophiles and psychrotrophs. Therefore, they grew well at all temperature conditions used in this study (Figs. 1 and 2), and outcompeted SE during growth under 8 and 10°C.

The one-step integrated data analysis and curve-fitting was previously used to study the growth kinetics of foodborne pathogens in foods (Huang, 2015a, 2015b). This approach was again proven successful for analyzing the growth curves of both SE and BK observed in this

study. The regression analysis converged easily and quickly (Table 1), producing estimates of the parameters for the secondary model (a , T_0 , and m) and the primary model (Y_0 for each growth curve, and the global $Y_{\max}$) for both SE (Table 2) and BK (Table 3). In Table 1, the mean square error (MSE) is 0.914 for SE, and 1.33 for BK. For SE, the RMSE is 0.964 Ln CFU/g, or 0.42 Log CFU/g. For BK, the RMSE is 1.16 Ln CFU/g, or 0.50 Log CFU/g. Fig. 1 clearly shows that the regression curves fit well for both SE (Column A) and BK (Column B).

For SE, except at 10°C, the bacteria grew well under all incubation temperatures and full growth curves, including all lag, exponential, and stationary phases, were observed (Fig. 1, Column A). According to the results of nonlinear regression (Table 2), the estimated maximum concentration ($Y_{\max}$) for SE in potato salad was 22.87 Ln CFU/g, or 9.93 Log CFU/g. The estimated nominal growth temperature (T_0) 5.19°C for SE. For background microorganisms, the bacteria grew well under all incubations temperatures (Fig. 1, Column B). According to Table 3, the maximum concentration ($Y_{\max}$) for BK was 21.36 Ln CFU/g, or 9.28 Log CFU/g. The estimated nominal growth temperature (T_0) for BK was 0.25°C, which is lower than that of SE.

Fig. 2 shows the effect of temperature on the specific growth rates and lag times of SE and BK in potato salad. It is apparent from this figure that the growth rate of BK is higher than that of SE between 0 and 37°C, as suggested by the Ratkowsky square-root model, whereas the lag time of the background microorganisms is lower than that of SE within the same temperature range, suggesting that the background microorganisms can grow better in potato salad than SE.

3.2 Model validation

Most models used in predictive microbiology are obtained by regression, and require validation to verify if the models are accurate using independent data sets. In the studies reported by Huang (2015a, 2015b), only one isothermal curve was set aside to validate the models developed using the one-step approach. In this study, two independent replicates of isothermal experiments were conducted to observe the bacterial growth and develop growth curves. However, only the data from one of the two replicates was used to determine the kinetic parameters of the growth of SE and BK. Since the one-step approach attempts to analyze the growth data across the entire temperature range used in this study, it is possible to find the kinetic parameters that best fitted the primary and secondary models by minimizing the global errors (RSS). Therefore, if the quality of growth data is sufficiently good, it is possible to use just one replicate of data in data analysis. However, the accuracy of the kinetic parameters must be validated before these parameters and the associated primary and secondary models can be used for making predictions.

In this study, the growth data obtained from the second replicate of the experiments was not used in model development. Therefore, this set of data was suitable for use to validate the kinetic models. Different from the studies previously reported by Huang (2015a, 2015b), the kinetic parameters and growth models were validated using multiple growth curves in this study. Fig. 3 shows the comparison between the experimentally observed growth curves and the corresponding growth curves calculated from the models using the kinetic parameters derived from this study.

Again, the growth of SE was clearly inhibited by the background microorganisms at 8 and 10°C after about ~220 h during incubation (Fig. 3, Column A), as the population of SE stopped increasing after this time point. The growth curve of SE obtained at 8°C was not used in

model development. However, the growth of SE was accurately predicted by the models when the incubation time was < 220 h. The growth of SE at 10°C was also accurately predicted when the incubation time was < 220 h. At temperatures above 15°C, the growth calculated by the models matched closely to the observed growth curves of SE. Excluding the predicted values of SE when $t > 220$ h at 8 and 10°C, the RMSE of the predictions was only 0.40 Log CFU/g for SE in potato salad. The distribution of the residual errors followed a Laplace distribution (location = 0.00, scale = 0.40), with 83.3% of the residual errors ranging between -0.5 and 0.5 Log CFU/g for predictions (Fig. 4A). With a Laplace distribution, the probability of observing ε decreases exponentially as ε increases.

For background microorganisms, the bacterial growth calculated using the models developed in the previous section also matched with the growth data under all incubation temperatures (Fig. 3, Column B). The RMSE of the predictions for BK was 0.66 Log CFU/g. The distribution of the residual errors also followed a logistic distribution (location = 0.025, scale = 0.30), with 68.3% of the residual errors ranging between -0.5 and 0.5 Log CFU/g of the prediction (Fig. 4B). Again, the background microorganisms grew better at 8 and 10°C and outcompeted the growth of SE under these temperature conditions (Fig. 3, Column B).

4. Conclusions

This study was conducted to evaluate the growth of SE in potato salad and use the observed growth curves to determine kinetic parameters of both primary and secondary models. This study applied a recently developed one-step kinetic analysis approach to analyze the growth curves. Duplicated experiments were conducted to observe the growth curves of both SE and

BK. The growth data of SE and BK from one of the duplicated experiments were used to develop growth models and determine the associated kinetic parameters, while the data from the secondary replicate were used to validate the accuracy of the growth models and kinetic parameters. The results clearly showed that the ones-step approach can be used to study the growth kinetics of both SE and BK, with the RSME of predictions only 0.40 Log CFU/g for SE and 0.66 Log CFU/g for BK, clearly suggesting that the kinetic models and kinetic parameters were very accurate in predicting the growth of both SE and BK in potato salad. The kinetic models and parameters of SE obtained in this study can be used to predict its growth in potato salad during storage and temperature abuse and to conduct risk assessment of SE if the product is contaminated. The growth models for BK developed in this study can be used to estimate the microbial shelf-life of potato salad. Since the growth models are validated using independent growth curves, they can be directly used in estimating the growth of SE and determining shelf-life of potato salad.

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Table 1. Results of nonlinear regression of the growth curves (Y, or natural logarithm of bacterial counts, in Eq. (1)) of *Salmonella* Enteritidis (SE) and background microorganisms (BK) in potato salad.

SE						
Source	DF	Sum of squares	Mean square	<u>RMSE</u>	F-value	Approx Pr > F
Model	10	2238.5	223.9		244.8	<0.0001
Error	65	59.5	0.914	<u>0.964¹</u>		
Corrected total	75	2298.0				
BK						
Source	DF	Sum of squares	Mean square	<u>RMSE</u>	F-value	Approx Pr > F
Model	10	2278.4	227.8		171.1	< 0.0001
Error	68	90.6	1.33	<u>1.16²</u>		
Corrected total	78	2369.0				

¹. 0.42 in Log CFU/g for RMSE.

². 0.50 in Log CFU/g for RMSE.

Table 2. Estimated parameters of the primary and secondary models for *Salmonella* Enteritidis (SE) in potato salad.

Parameter	Estimate	Approx. Std. Error	95% confident limits	
			Lower	Upper
T_0	5.19	0.46	4.27	6.12
a	0.0412	0.0013	0.0383	0.0436
<u>m</u>	1.090	0.237	0.617	1.563
$Y_{0, 10^*}$	$8.88^1 (3.86^2)$	0.64 (0.28)	7.61 (3.30)	10.15 (4.41)
$Y_{0, 15}$	10.02 (4.35)	0.50 (0.22)	9.02 (3.92)	11.02 (4.79)
$Y_{0, 20}$	9.05 (3.93)	0.42 (0.18)	8.21 (3.57)	9.88 (4.29)
$Y_{0, 25}$	9.63 (4.18)	0.41 (0.18)	8.80 (3.82)	10.46 (4.54)
$Y_{0, 30}$	9.14 (3.97)	0.40 (0.17)	8.34 (3.62)	9.93 (4.31)
$Y_{0, 33}$	9.27 (4.03)	0.40 (0.17)	8.47 (3.42)	10.07 (4.19)
$Y_{0, 37}$	8.76 (3.81)	0.45 (0.19)	7.87 (3.42)	9.65 (4.19)
$Y_{\max}$	22.87(9.93)	0.20 (0.09)	22.46 (9.75)	23.27 (10.10)

* The initial concentration Y_0 . The second numerical subscripts of Y_0 are incubation temperatures.

¹. Bacterial counts in Ln CFU/g.

². bacterial counts in Log CFU/g

Table 3. Estimated parameters of the primary and secondary models for background microorganism (BK) in potato salad.

Parameter	Estimate	Approx. Std. Error	95% confident limits	
			Lower	Upper
T_0	0.25	1.20	-2.13	2.63
a	0.0372	0.002	0.0329	0.0412
<u>m</u>	1.509	0.314	0.882	2.135
$Y_{0.10^*}$	$8.37^1 (3.64^2)$	1.08 (0.47)	6.21 (2.70)	10.54 (4.58)
$Y_{0.15}$	6.84 (2.97)	0.67 (0.29)	5.50 (2.39)	8.18 (3.55)
$Y_{0.20}$	7.80 (3.19)	0.54 (0.23)	6.73 (2.92)	8.87 (3.85)
$Y_{0.25}$	8.21 (3.56)	0.53 (0.23)	7.15 (3.10)	9.26 (4.02)
$Y_{0.30}$	7.25 (3.15)	0.51 (0.22)	6.23 (2.70)	8.27 (3.59)
$Y_{0.33}$	8.36 (3.63)	0.53 (0.23)	7.31 (3.18)	9.41 (4.09)
$Y_{0.37}$	7.94 (3.45)	0.57 (0.25)	6.80 (2.95)	9.07 (3.94)
$Y_{\max}$	21.37 (9.28)	0.20 (0.09)	20.97 (9.11)	21.76 (9.45)

* The initial concentration Y_0 . The second numerical subscripts of Y_0 are incubation temperatures.

¹. Bacterial counts in Ln CFU/g.

². bacterial counts in Log CFU/g.

Figure 1. One-step curve fitting of multiple growth curves. Column A: SE; Column B: BK.
 SED: SE growth data; SEM: SE growth model. BKD: BK growth data; BKM: BK growth
 model.

Figure 2. Effect of temperature on growth rate and lag time obtained from one-step analysis. A. Correlation between $\mu_{\max}$ and incubation temperature (Ratkowsky square-root model). B. Effect of temperature on λ .

Figure 3. Validation of mathematical models. Column A: SE; Column B: BK. SED: SE growth data; SEM: SE growth model. BKD: BK growth data; BKM: BK growth model. SEM: SE growth model. BKD: BK growth data; BKM: BK growth model.

408 Figure 4. The distribution of residual errors of prediction. A: SE; B: BK.

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