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Development of Predictive Mathematical Model for the Growth Kinetics of *Staphylococcus aureus* by Response Surface Model

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Abstract A response surface model was developed for predicting the growth rates of *Staphylococcus aureus* in tryptic soy broth (TSB) medium as a function of combined effects of temperature, pH, and NaCl. The TSB containing six different concentrations of NaCl (0, 2, 4, 6, 8, and 10%) was adjusted to an initial of six different pH levels (pH 4, 5, 6, 7, 8, 9, and 10) and incubated at 10, 20, 30, and 40°C. In all experimental variables, the primary growth curves were well (r^2=0.9000 to 0.9975) fitted to a Gompertz equation to obtain growth rates. The secondary response surface model for natural logarithm transformations of growth rates as a function of combined effects of temperature, pH, and NaCl was obtained by SAS's general linear analysis. The predicted growth rates of the *S. aureus* were generally decreased by basic (pH 9–10) or acidic (pH 5–6) conditions and higher NaCl concentrations. The response surface model was identified as an appropriate secondary model for growth rates on the basis of correlation coefficient (r=0.9703), determination coefficient (r^2=0.9415), mean square error (MSE=0.0185), bias factor (B_f=1.0216), and accuracy factor (A_f=1.2583). Therefore, the developed secondary model proved reliable for predictions of the combined effect of temperature, NaCl, and pH on growth rates for *S. aureus* in TSB medium.

Keywords: *Staphylococcus aureus*, response surface model, growth rates

Staphylococcus aureus is one of the leading causes of foodborne disease as well as one of the most prevalent causes of microbial foodborne diseases worldwide [19, 26]. *S. aureus* was determined to be the etiological agent in 367 (19.6%) of 1,869 documented bacterial foodborne diseases in the United States [2]. Approximately 25 major outbreaks of Staphylococcal food poisoning disease occur annually in the United States [17]. *S. aureus* has been estimated by the Centers for Disease Control and Prevention to cause 185,060 illness, 1,753 hospitalizations, and 2 deaths per years in the United States [24]. Because of its predominance as a food poisoning microorganism, *S. aureus* has been extensively studied to determine the physical and chemical parameters that affect its growth and toxin formation [3, 4, 18, 19].

Control of microbial foodborne diseases in many types of food relies on a combination of barrier factors, none of which are present at levels sufficient by themselves to inhibit microorganisms. Under these environments, mathematical models provide an essential tool of predicting microbial growth [20, 21, 40].

Impressive progress is being made in predictive microbiology, and models are increasingly becoming standard research tools and being helpful in the evaluation and design of food-processing procedures. Use of predictive models may eventually play a highly effective role in the prevention of food poisoning. These models as a function of main environmental controlling factors will improve the shelf-life and safety of foods [6–8, 19, 27, 30, 36–38, 41, 42]. There are several developed mathematical quantitative models of *S. aureus* growth in predictive food microbiology. However, no predictive models have been constructed describing the effect of temperature in combination with NaCl and acidic to basic pH.

Therefore, the objective of this study was to investigate the combined effects of temperature, NaCl, and pH on the

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growth kinetics of *S. aureus* in a broth system with the goal of developing a model that could be used to predict the maximum growth rates of the organisms in any combination of the variables.

MATERIALS AND METHODS

Bacterial Culture

Staphylococcus aureus ATCC 35556 was used in the study. Tryptic soy broth (TSB, Difco Laboratories, Detroit, MI, U.S.A.) was used for maintenance and growth of the bacterial strain.

Experimental Design

A central composite design was used, incorporating the following variables and levels. The 168 factor combinations were tested. The response surface model was developed for predicting the growth rates of *Staphylococcus aureus* in tryptic soy broth (TSB) medium as a function of combined effects of temperature, pH, and NaCl. The TSB containing six different concentrations of NaCl (0, 2, 4, 6, 8, and 10%) was adjusted to an initial of six different pH levels (pH 4, 5, 6, 7, 8, 9, and 10) and incubated at 10, 20, 30, and 40°C.

Preparation and Inoculation of Culture Media

TSB containing six different concentrations of NaCl (0, 2, 4, 6, 8, and 10% w/v) was autoclaved at 121°C for 15 min and allowed to cool. The pH of the media was then adjusted to an initial of 4, 5, 6, 7, 8, 9, or 10 using 1 N NaOH or 1 N HCl solution. Microplate wells were filled with 150 μl of each media condition to which 50 μl of inoculums containing 10^7 CFU/ml of *S. aureus* was added. Control wells containing 200 μl of uninoculated medium were used as blanks and also to check the sterility of the medium.

Growth Temperature and Growth Rate Measurements

Growth rates of *S. aureus* in microplate wells incubate d at 10, 20, 30, and 40°C were measured every 2 h as optical density at 600 nm by an Automated Microplate Reader (ELx808, Biotech Ltd., Winooski, VI, U.S.A.), using Microplate Data Analysis Software. The observance values were natural log-transformed to homogenize variances.

Primary Modeling

Growth curves of the resulting absorbance versus time values were iteratively generated using the Gompertz equation and fit to a nonlinear regression model (Prism, version 4.0, GraphPad Software, San Diego, CA, U.S.A.) to determine maximum growth rates (GR, in $\log_{10}$ CFU/ml/h) at each incubation temperature.

$$Y=N_0+C*\exp(\exp((2.718*GR/C)*(LT-X)+1))$$

The Gompertz parameter values were log cell number (Y), incubation time (X), log initial number of cells (N_0), difference between initial and final cell numbers (C), lag time before growth (LT), and maximum growth rates (GR), as described by Gibson *et al.* [14].

Secondary Modeling

The response surface model in terms of temperature, sodium chloride concentration, and pH was calculated on the growth rates. The Gompertz parameter for *S. aureus* growth data was determined by the least-squares analysis of PROC GLM of the SAS version 8.1 [35].

$$\ln \text{Growth Rates}=b_0+b_1A+b_2B+b_3C+b_4A^2+b_5B^2+b_6C^2+b_7AB+b_8AC+b_9BC+\varepsilon$$

The response surface model parameter values were incubation temperature (A), initial pH (B), sodium chloride concentration (C), regression coefficients (b_0–b_9), and random error (ε), as described by Gibson *et al.* [14].

Evaluation of Model Performance

The correlation coefficient (r) and determination coefficient (r^2) provided by GraphPad [15] is often used as an overall measure of the prediction attained. It measures the fraction of the variation about the mean that is explained by a model.

The mean square error (MSE), the residual sum of squares divided by the number of degrees of freedom, is a measure of variability remaining, which is not accounted for by deliberate changes in factors such as temperature, pH, and a_w.

$$MSE=(\sum \log(\text{predicted growth rates}/\text{observed growth rates})^2)/\text{number of observations}$$

The bias factors (B_f) answer the question whether, on average, the observed values lie above or below the line equivalence and, if so, by how much. It gives the structural deviations of a model.

$$B_f=10^{(\sum \log(\text{predicted growth rates}/\text{observed growth rates}))/\text{number of observations}}$$

The accuracy factor (A_f) averages the distance between each point and the line of equivalence as a measure of how close, on average, predictions are to observe.

$$A_f=10^{\sum |\log(\text{predicted growth rates}/\text{observed growth rates})|/\text{number of observations}}$$

RESULTS AND DISCUSSION

Primary Modeling

Absorbance measurement is regarded as an alternative method of viable count measurement because the traditional

viable count measurement for collecting the growth data are time-consuming and labor intensive, However, the absorbance measurement is typically used to determine only growth rates [10, 11, 25, 26, 43] owing to its high detection levels, which make it difficult to measure the lag time [24]. Therefore, the absorbance measurement was used for the development of a predictive growth model for the growth rates of *S. aureus* in TSB medium in the current study.

The growth rates of *S. aureus* in the seven different pH levels of TSB medium in the presence of six different concentrations of NaCl at the incubation temperature of 10, 20, 30, and 40°C are shown in Tables 1, 2, 3, and 4. No growth of *S. aureus* was observed in the combination of all experimental variables of either 10% NaCl or pH 4 at the incubation of 10, 20, 30, and 40°C. At the incubation temperature of 10°C, the growth of *S. aureus* was not observed in 0, 2%, 4% NaCl of pH 4, in NaCl 6% of pH 4, 5, and in 8%, 10% NaCl in TSB medium. At the incubation temperature of 20°C, the growth of *S. aureus* was not observed in 0, 2%, 4%, 6% NaCl of pH 4, in 8% NaCl of pH 4, 9, 10, and in 10% NaCl in TSB medium. At the incubation temperature of 30°C, the growth of *S. aureus* was not observed in 0% NaCl of pH 10, in 2%, 4%, 6% NaCl of pH 4, in 8% NaCl of pH 4, 9, 10, and in 10% NaCl in TSB medium. At the incubation temperature of 40°C, the growth of *S. aureus* was not observed in 0, 2% NaCl of pH 4, 10, in 4%, 6% NaCl of pH 4, and in 8% NaCl of pH 4, 5, 9, 10 and in 10% NaCl in TSB medium. Therefore, the model development of the current study involved 103 growth curves conducted with 103 combinations of temperature, NaCl, and pH in TSB medium.

The Gompertz equation is typically used to fit bacterial growth curves for estimating lag time and maximum growth rates in the U.S. Department of Agriculture [5, 8, 9, 29]. Therefore, the current study used the Gompertz equation to fit growth curves for *S. aureus*. Best-fit values of growth rates on 103 growth curves in the primary model are also shown in Tables 1, 2, 3, and 4. The data of growth rates for TSB medium fitted the Gompertz equation model well, with a high degree of goodness of fits (r^2=0.900 to 0.9975) at all treatment factors (Tables 1, 2, 3, and 4).

Secondary Modeling

In the current study, the model development phase of this involved 103 growth curves conducted on fewer than 168 combinations of temperature, NaCl, and acidic to basic pH in TSB medium. The growth rates from these 103 growth curve fits were transformed to their natural logarithm to stabilize model variance [14] and were subjected to response surface analysis using SAS's general linear model. The following equation was given:

Table 1. Best-fit growth rates (GR) of *Staphylococcus aureus* in TSB medium incubated at 10°C for the primary modeling.

T (°C)	NaCl (%)	pH	GR (h^{-1})	r^{2a}	T (°C)	NaCl (%)	pH	GR (h^{-1})	r^2
10	0	4	NG[b]	NA[c]	10	6	4	NG	NA
10	0	5	0.000	0.995	10	6	5	NG	NA
10	0	6	0.010	0.947	10	6	6	0.013	0.947
10	0	7	0.035	0.982	10	6	7	0.027	0.907
10	0	8	0.044	0.944	10	6	8	0.025	0.971
10	0	9	0.036	0.938	10	6	9	0.005	0.952
10	0	10	0.012	0.950	10	6	10	NG	NA
10	2	4	NG	NA	10	8	4	NG	NA
10	2	5	0.000	0.998	10	8	5	NG	NA
10	2	6	0.030	0.949	10	8	6	NG	NA
10	2	7	0.052	0.984	10	8	7	NG	NA
10	2	8	0.057	0.951	10	8	8	NG	NA
10	2	9	0.045	0.945	10	8	9	NG	NA
10	2	10	0.017	0.990	10	8	10	NG	NA
10	4	4	NG	NA	10	10	4	NG	NA
10	4	5	0.000	0.994	10	10	5	NG	NA
10	4	6	0.031	0.954	10	10	6	NG	NA
10	4	7	0.049	0.952	10	10	7	NG	NA
10	4	8	0.050	0.942	10	10	8	NG	NA
10	4	9	0.035	0.953	10	10	9	NG	NA
10	4	10	0.003	0.978	10	10	10	NG	NA

[a] r^2, coefficient of determination.
[b] NG, no growth.
[c] NA, no application.

Table 2. Best-fit growth rates (GR) of *Staphylococcus aureus* in TSB medium incubated at 20°C for the primary modeling.

T (°C)	NaCl (%)	pH	GR (h^{-1})	r^{2a}	T (°C)	NaCl (%)	pH	GR (h^{-1})	r^2
20	0	4	NG[b]	NA[c]	20	6	4	NG	NA
20	0	5	0.106	0.992	20	6	5	0.062	0.980
20	0	6	0.147	0.977	20	6	6	0.091	0.954
20	0	7	0.172	0.979	20	6	7	0.105	0.967
20	0	8	0.180	0.934	20	6	8	0.101	0.937
20	0	9	0.171	0.953	20	6	9	0.081	0.910
20	0	10	0.146	0.951	20	6	10	0.044	0.914
20	2	4	NG	NA	20	8	4	NG	NA
20	2	5	0.111	0.984	20	8	5	0.008	0.913
20	2	6	0.148	0.989	20	8	6	0.034	0.963
20	2	7	0.169	0.980	20	8	7	0.043	0.975
20	2	8	0.173	0.947	20	8	8	0.036	0.981
20	2	9	0.161	0.967	20	8	9	NG	NA
20	2	10	0.132	0.926	20	8	10	NG	NA
20	4	4	NG	NA	20	10	4	NG	NA
20	4	5	0.096	0.994	20	10	5	NG	NA
20	4	6	0.129	0.961	20	10	6	NG	NA
20	4	7	0.146	0.982	20	10	7	NG	NA
20	4	8	0.147	0.947	20	10	8	NG	NA
20	4	9	0.131	0.948	20	10	9	NG	NA
20	4	10	0.098	0.912	20	10	10	NG	NA

[a]r^2, coefficient of determination.
[b]NG, no growth.
[c]NA, no application.

Table 3. Best-fit growth rates (GR) of *Staphylococcus aureus* in TSB medium incubated at 30°C for the primary modeling.

T (°C)	NaCl (%)	pH	GR (h^{-1})	r^{2a}	T (°C)	NaCl (%)	pH	GR (h^{-1})	r^2
30	0	4	0.202	0.971	30	6	4	NG[b]	NA[c]
30	0	5	0.259	0.990	30	6	5	0.155	0.992
30	0	6	0.299	0.993	30	6	6	0.184	0.955
30	0	7	0.323	0.995	30	6	7	0.196	0.978
30	0	8	0.330	0.970	30	6	8	0.192	0.972
30	0	9	0.320	0.943	30	6	9	0.171	0.980
30	0	10	NG	NA	30	6	10	0.133	0.977
30	2	4	NG	NA	30	8	4	NG	NA
30	2	5	0.243	0.990	30	8	5	0.081	0.996
30	2	6	0.280	0.995	30	8	6	0.107	0.992
30	2	7	0.300	0.994	30	8	7	0.115	0.992
30	2	8	0.303	0.980	30	8	8	0.107	0.988
30	2	9	0.290	0.945	30	8	9	NG	NA
30	2	10	0.260	0.922	30	8	10	NG	NA
30	4	4	NG	NA	30	10	4	NG	NA
30	4	5	0.209	0.985	30	10	5	NG	NA
30	4	6	0.242	0.926	30	10	6	NG	NA
30	4	7	0.258	0.925	30	10	7	NG	NA
30	4	8	0.257	0.900	30	10	8	NG	NA
30	4	9	0.240	0.918	30	10	9	NG	NA
30	4	10	0.206	0.909	30	10	10	NG	NA

[a]r^2, coefficient of determination.
[b]NG, no growth.
[c]NA, no application.

Table 4. Best-fit growth rates (GR) of *Staphylococcus aureus* in TSB medium incubated at 40°C for the primary modeling.

T (°C)	NaCl (%)	pH	GR (h^{-1})	r^{2a}	T (°C)	NaCl (%)	pH	GR (h^{-1})	r^2
40	0	4	NG[b]	NA[c]	40	6	4	NG	NA
40	0	5	0.425	0.987	40	6	5	0.262	0.985
40	0	6	0.465	0.991	40	6	6	0.290	0.957
40	0	7	0.488	0.986	40	6	7	0.301	0.963
40	0	8	0.494	0.943	40	6	8	0.296	0.959
40	0	9	0.483	0.980	40	6	9	0.274	0.972
40	0	10	NG	NA	40	6	10	0.236	0.977
40	2	4	NG	NA	40	8	4	NG	NA
40	2	5	0.390	0.989	40	8	5	NG	NA
40	2	6	0.426	0.968	40	8	6	0.193	0.988
40	2	7	0.445	0.973	40	8	7	0.201	0.984
40	2	8	0.447	0.936	40	8	8	0.191	0.986
40	2	9	0.433	0.925	40	8	9	NG	NA
40	2	10	NG	NA	40	8	10	NG	NA
40	4	4	NG	NA	40	10	4	NG	NA
40	4	5	0.336	0.991	40	10	5	NG	NA
40	4	6	0.368	0.955	40	10	6	NG	NA
40	4	7	0.383	0.961	40	10	7	NG	NA
40	4	8	0.381	0.945	40	10	8	NG	NA
40	4	9	0.363	0.924	40	10	9	NG	NA
40	4	10	0.329	0.969	40	10	10	NG	NA

[a]r^2, coefficient of determination.
[b]NG, no growth.
[c]NA, no application.

$$\ln \text{Growth Rate} = -0.62155 + (0.01221 * \text{Temp}) + (0.13445 * \text{pH}) + (0.03645 * \text{NaCl}) + (0.00007 * \text{Temp}^2) + (-0.00831 * \text{pH}^2) + (-0.00242 * \text{NaCl}^2) + (-0.00009 * \text{Temp} * \text{pH}) + (-0.00099 * \text{Temp} * \text{NaCl}) + (-0.00191 * \text{pH} * \text{NaCl})$$

This equation was identified as an appropriate secondary model for growth rates on the basis of correlation coefficient ($r=0.9703$) and determination coefficient ($r^2=0.9415$). This equation estimated the predicted growth rate of *S. aureus* in combinations of temperature, NaCl, and pH in TSB medium, shown in Figs. 1, 2, 3, and 4. When the overall main effects of NaCl concentrations or pH levels in TSB medium incubated at 10, 20, 30, and 40°C were compared, the predicted growth rates of the *S. aureus* were generally decreased by either basic (pH 9–10) or acidic (pH 5–6) conditions and

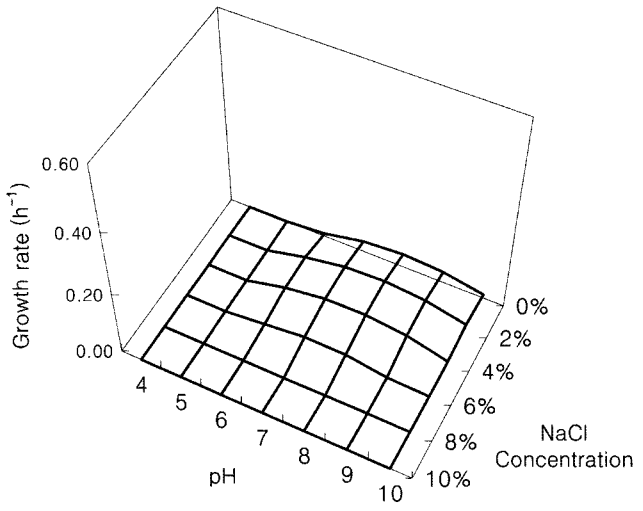

Fig. 1. Predicted growth rates of *Staphylococcus aureus* in the presence of sodium chloride in different pH levels of TSB medium incubated at 10°C.

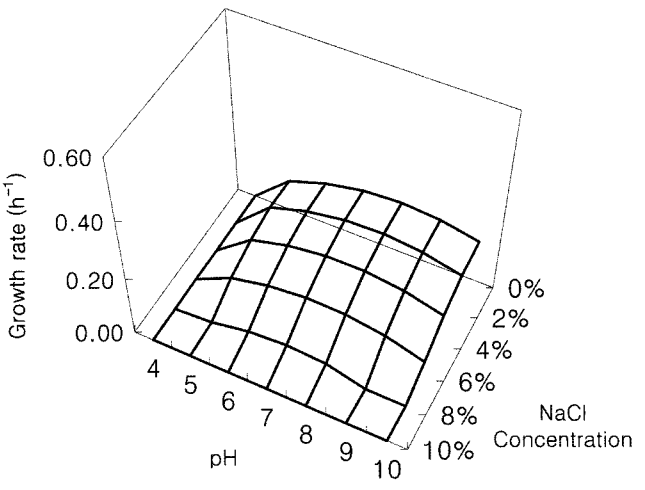

Fig. 2. Predicted growth rates of *Staphylococcus aureus* in the presence of sodium chloride in different pH levels of TSB medium incubated at 20°C.

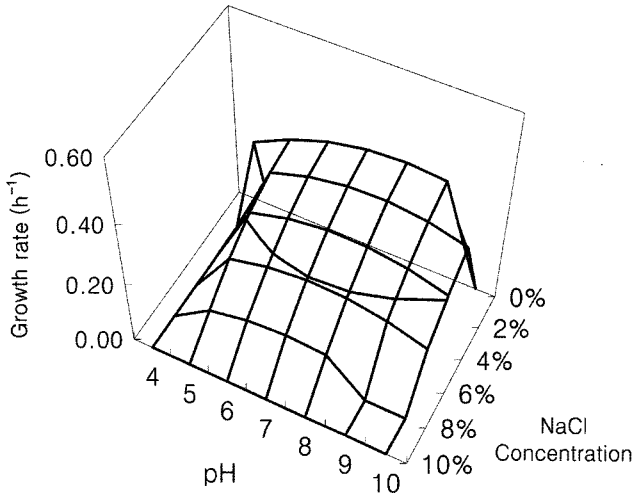

Fig. 3. Predicted growth rates of *Staphylococcus aureus* in the presence of sodium chloride in different pH levels of TSB medium incubated at 30°C.

higher NaCl concentrations (Figs. 1, 2, 3, and 4). However, the predicted growth rates of the *S. aureus* was not inhibited at pH 8 compared with at pH 7 of TSB medium incubated at 10, 20, 30, and 40°C. Although the effects of the incubation temperature for the predicted growth rates were not compared, the predicted growth rates in the combination of all experimental variables appeared to be generally less at the storage of 10°C than that of 20, 30, and 40°C.

Evaluation of the Model Performance

Table 5 presents four different statistical indices of the secondary modeling step for the predicted growth rates of *S. aureus* in TSB medium. The higher the value of r^2, the better is the prediction by the model [12, 16, 39]. The lower

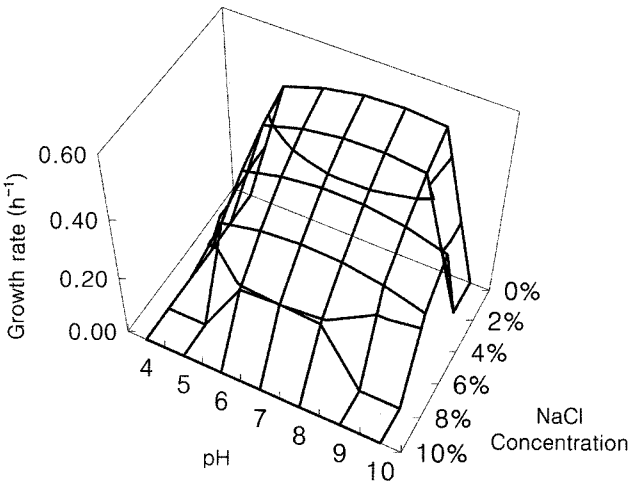

Fig. 4. Predicted growth rates of *Staphylococcus aureus* in the presence of sodium chloride in different pH levels of TSB medium incubated at 40°C.

Table 5. Statistical indices of the secondary response surface modeling step for growth rates of *Staphylococcus aureus* in TSB medium.

Model	r[a]	r^{2b}	MSE[c]	B_f[d]	A_f[e]
Response surface model	0.9703	0.9415	0.0185	1.0216	1.2583

[a]r, correlation of coefficient.
[b]r^2, coefficient of determination.
[c]MSE, mean square error.
[d]B_f, bias factor.
[e]A_f, accuracy factor.

the value of MSE, the better is the adequacy of the model to describe the data [1, 39]. $B_f<1$ indicates a "fail safe" model [32]. $B_f>1$ indicates a "fail dangerous" model [32]. Ross [33] also noted that for models describing pathogen growth rates, B_f in the range of 0.9–1.05 could be considered good, in the range of 0.7–0.9 or 1.06–1.15 considered acceptable, and <0.7 or >1.5 considered unacceptable. The larger the value of A_f, the less accurate is the average estimate. An acceptable model that predicts the growth rates of *Listeria monocytogenes* as a function of temperature, NaCl, and pH could be expected to have A_f in the range of 1.3–1.5 [31]. If there is a display of $A_f=B_f=1$, the predictive model is perfect.

Based on the above statements about the four different statistical indices, our results indicated that the developed response surface model proved reliable predictions of the combined effects of temperature, NaCl, and pH on the growth rates of *S. aureus* in TSB medium.

However, for risk management, further work is necessary to confirm the prediction of the growth rate model for *S. aureus* in food products. Furthermore, there is an urgent necessity in developing models of growth and death, and survival and transmission of *S. aureus* occurs in diverse food matrices and food processing plants exposed to various environmental conditions. Therefore, the developed model of growth rates will reduce the uncertainty against *S. aureus* in the food production, processing, and distribution processes and thus will ensure food safety.

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