Food Sci. Biotechnol. 19(3): 763-768 (2010)
DOI 10.1007/s10068-010-0107-x

RESEARCH ARTICLE

Mathematical Modeling on the Growth of *Staphylococcus aureus* in Sandwich

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Received: 18 January 2010 / Revised: 2 March 2010 / Accepted: 15 March 2010 / Published Online: 30 June 2010

Abstract The growth of *Staphylococcus aureus* in sandwich fillings at different incubation temperatures was tested. These growth data were fitted into the Gompertz model, Logistic model, and Baranyi model in order to compare the goodness-of-fit of the 3 primary models using several factors such as coefficient of determination (R^2), the standard deviation ($S_{y.x}$), and the Akaike's information criterion (AIC). The Gompertz model showed the best statistical fit. Hence, growth parameters such as specific growth rate (SGR) and lag time (LT) obtained from the Gompertz model were used to construct the secondary models. Further, developed models were evaluated by bias factor (B_f) and accuracy factor (A_f). For the SGR, the B_f value was 0.993 and A_f value was 1.156 which indicated conservative predictions. While for LT, a clear deviation was observed between predictions and observations (B_f=0.635 and A_f=1.592). The results, however, were also considered acceptable after comparing with previous publications.

Keywords: *Staphylococcus aureus*, primary model, secondary model, sandwich, evaluation

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Introduction

Staphylococcus aureus, first reported by Rosenbach in 1884, is a Gram-positive, pathogenic microorganism (1). It is the most common cause of staph infections such as pimples, impetigo, meningitis, osteomyelitis, endocarditis, and septicemia. It is able to secrete several different kinds of toxins which are associated with specific diseases. It was reported by Centers for Disease Control and Prevention that the consumption of food contaminated with *S. aureus* results in 185,060 illnesses, 1,753 hospitalizations, and 2 deaths annually in the USA, mostly occurring among the elderly, in infants, and in ill people (2). Therefore, *S. aureus* is considered responsible for one of the most common types of food poisoning (3), especially for the following food products, meat and meat products, poultry and egg products, salads, bakery products, sandwich fillings, and milk and dairy products (4). Sandwich is a popular type of food all over the world consisting of two or more slices of bread which is filled with a combination of salad vegetables, meat, ham, cheese, and other various sauces.

Predictive microbiology is a promising and rapidly developing area of food microbiology, which combines mathematics, engineering, chemistry, and microbiology to give microbial behavioral predictions in specific food products under defined conditions (5,6). It is perceived as a useful tool in planning hazard analysis critical control point (HACCP) programs, making decisions, and regulating plans and policies for the food industry, as they provide the first estimates of expected changes in microbial populations when exposed to a specific set of conditions (7,8). Predictive models have two main advantages. One is to predict the remaining shelf-life of a particular type of food product at different temperatures or reference conditions

during processing. The other one is to devise a preservation strategy for the sake of restraining the growth of pathogenic microorganisms to an acceptable extent (9). Generally, predictive models include a primary model, a secondary model and a tertiary model. Primary models are used to describe the functions of the microbial populations and time under constant environmental conditions (10), including the Gompertz model, Logistic model, Baranyi model, and Monod model, etc (11). Secondary models are used to describe the influences of environmental factors on growth parameters [e.g., specific growth rate (SGR), lag time (LT), and maximum population density (MPD)] obtained from primary models under dynamic conditions (12). Examples of these models include the response surface model, square root model, polynomial model, Ratkowsky model, and Arrhenium model. The tertiary models usually refer to developing software based on the primary models and secondary models such as pathogen modeling program (PMP), food spoilage predictor, and combined database (Combase) (11). Numerous studies have been published using a wide variety of models which indicate that different models have different performances. Juneja *et al.* (13) measured the goodness-of-fit of 3 primary models on growth of *Salmonella* in chicken. Ding *et al.* (14) found that the performance of the Gompertz model was better than the Logistic model and Baranyi model on growth of *Listeria monocytogenes* in lettuce. Also, Zhou *et al.* (15) compared the performances of the modified Gompertz model, Baranyi model, and the Logistic model for *Brochothrix thermosphacta* isolated from chilled pork in tryptone soya broth. However, few literatures have been focused on comparison study of different predictive models of *S. aureus*.

Consequently, the Gompertz model, Logistic model, and Baranyi model were selected in this study to describe the microbial growth of *S. aureus* in sandwich fillings. Several mathematical indices were used to compare the performances of the developed models to determine which was more reliable. Sequentially, secondary models were then established using the specific growth rate (SGR) and lag time (LT) obtained from the most suitable primary model.

Materials and Methods

Bacterial culture Three strains of *Staphylococcus aureus* (ATCC 13565, ATCC 14458, and ATCC 23235) obtained from the Korean National Institute of Health (Seoul, Korea) were used throughout this study. All strains were presented at –70°C in tryptic soy broth (TSB, Difco, Sparks, MD, USA) with a 0.6% yeast extract (YE, Difco) containing 20% glycerol. Each strain was transferred 10 μL of the stock culture into 10 mL of TSBYE, and then incubated at 35°C for 24 hr. Then the cells were harvested by centrifugation (5 min at 5,000×g) and washed twice in sterile distilled water. After a second washing, cocktails were adjusted by mixing equal volumes of the 3 bacterial suspensions of the inoculums diluted with 90 mL of sterile 0.1%(w/v) peptone water (Difco) to a final inoculum level of 5 log CFU/mL.

Inoculation of *S. aureus* in sandwich fillings Fresh Joe's sandwiches with the main fillings of ham and cheese were purchased from a local supermarket in Chuncheon, Korea. Sandwich samples were weighed every 10 g for 1 group and every group was inoculated by pipetting 0.1 mL of about 10^5 CFU/mL *S. aureus* suspension on to the surface of sample. The inoculated samples were then left on the clean bench for 3 hr until completely dry. This procedure resulted in initial pathogen inoculum levels of approximately 3.0 log CFU/g of sandwich.

Microbiological analysis Sandwich samples of 10.0±0.2 g were aseptically mixed with 90 mL of sterile 0.1% peptone water (Difco) in stomacher bags (Whirl-Pak; Nasco, Janesville, WI, USA) and transferred to a stomacher (Lab-blender 400; Seward, London, UK) to homogenize for 2 min at 200 rpm at room temperature. Further, 0.1 mL aliquots of the appropriate dilution were spread on the surface of duplicate plates of Baird-Parker agar base (Difco) supplemented with 50 mL of egg yolk Tellurite solution to enumerate the population of *S. aureus*, and the plates were then incubated at 37°C for 24 hr. After incubation, colonies of *S. aureus* were enumerated and expressed as log CFU/g.

Model development Growth data of *S. aureus* in sandwich fillings were collected at each storage temperature (7, 10, 12, 14, 16, 20, 25, and 30°C) and were then fitted into 3 primary models, Gompertz model (Eq. 1), Logistic model (Eq. 2), and Baranyi model (Eq. 3), using the GraphPad prism software (version 4, GraphPad Software, Inc., San Diego, CA, USA) and DMFit curve-fitting software (courtesy of J. Baranyi; Institute of Food Research, Norwich, UK).

$$N_t=N_0+C\exp\{\exp[(2.718\ \mu/C)(\lambda-t)+1]\} \quad (1)$$

$$N_t=N_0+C/\{1+\exp[(-2.718\ \mu/N_0)(t-\lambda+N_0/2.718\ \mu)\} \quad (2)$$

$$N_t=N_0+\mu f(t)-\ln\left(1+\frac{e^{\mu f(t)}-1}{e^{(N_{max}-N_0)}}\right) \quad (3)$$

$$f(t)=t+\frac{1}{\nu}\ln(e^{-\nu t}+e^{-\mu\lambda}-e^{(-\nu t-\mu\lambda)})$$

where N_t is the microbial counts in units of log CFU/g at time, t (day), N_0 is the logarithm of initial microbial counts

(log CFU/g), N_{max} is the logarithm of maximum microbial counts (log CFU/g), C is the difference between initial and final cell numbers, μ is the specific growth rate (log CFU/day), v is the rate of increase of the limiting substrate, assumed to be equal to μ and λ is the lag time (day).

Secondary models were developed for SGR (Eq. 4) and LT (Eq. 5), respectively, using SPSS 13.0 (Statistical Package for Social Science, Chicago, IL, USA).

$$\mu=[b(T-T_0)]^2 \quad (4)$$

$$\lambda=e^{b(T-T_0)} \quad (5)$$

where μ is the specific growth rate (log CFU/day), λ is the lag time (day), b is the regression constant, T is the temperature (°C) and T_0 is the minimum temperature required for growth.

Model evaluation Measures of coefficient of determination (R^2), the standard deviation ($S_{y.x}$, Eq. 6) and the Akaike's information criterion (AIC, Eq. 7) (13) were used to compare the goodness-of-fit of the 3 primary models selected in this study.

$$S_{y.x}=\sqrt{\frac{SS}{Df}} \quad (6)$$

$$AIC=n\times Ln\left(\frac{SS}{n}\right)+2p \quad (7)$$

where SS is the sum of squares in the model, Df is degrees of freedom which equal to number of data points minus the number of parameters fit, n is the number of observations, and p is the number of parameters in the model.

Performances of the secondary models were validated by several mathematical or statistical indices such as bias factor (B_f, Eq. 8) and accuracy factor (A_f, Eq. 9) as follows:

$$B_f=10^{\left(\sum_{i=1}^{n}\log(obs/pred)/n\right)} \quad (8)$$

$$A_f=10^{\left(\sum_{i=1}^{n}|\log(obs/pred)|/n\right)} \quad (9)$$

where n is the number of observations, $pred$ is the predicted SGR or LT value, and obs is the observed SGR or LT value.

Results and Discussion

Primary modeling of *S. aureus* The growth data of *S. aureus* in sandwich fillings obtained at different storage temperatures (7, 10, 12, 14, 16, 20, 25, and 30°C) were fitted into the Gompertz model, Logistic model, and Baranyi model using the GraphPad prism software and DMFit curve-fitting software, respectively. The goodness-of-fit and statistical characteristics of the 3 types of primary models were then compared employing coefficient of determination (R^2), the standard deviation ($S_{y.x}$) and the Akaike's information criterion (AIC). Figure 1 illustrates the comparison of coefficient of determination (R^2) of the Gompertz model, Logistic model, and Baranyi model. R^2 is a fraction between 0.0 and 1.0, which is regarded as a valuable tool to quantify the goodness-of-fit of models. In general, higher R^2 value means that particular model fit the data better (16,17). As shown in Fig. 1, lowest R^2 values were observed from the Baranyi model at each storage temperature, followed by ones obtained from the Logistic model and the Gompertz model. The results indicate that the Gompertz model is able to provide a better statistical fit than the other two. The value of $S_{y.x}$ is the standard deviation of the vertical distances of the points from the line. The lower $S_{y.x}$ value indicates a better agreement between observed values and predicted values. A comparison of the $S_{y.x}$ calculated from the three primary models is presented in Fig. 2. It can be observed that $S_{y.x}$ values obtained from

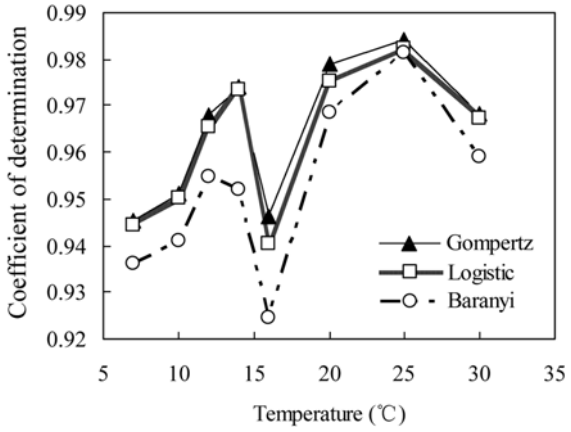

Fig. 1. Comparison of coefficient of determination (R^2) of the Gompertz model, Logistic model, and Baranyi model.

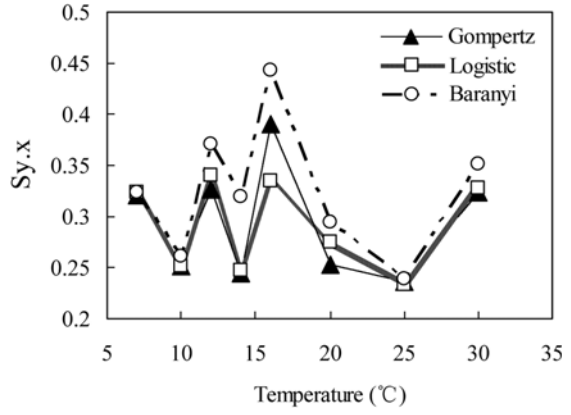

Fig. 2. Comparison of the standard deviation ($S_{y.x}$) of the Gompertz model, Logistic model, and Baranyi model.

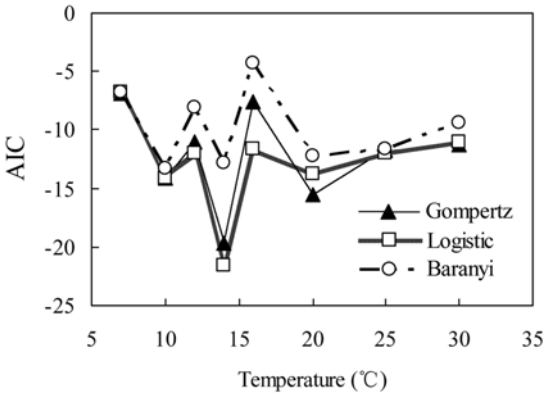

Fig. 3. Comparison of Akaike's information criterion (AIC) of the Gompertz model, Logistic model, and Baranyi model.

the Gompertz model at different storage temperatures is lower than ones from the other 2 models, which demonstrate that the Gompertz model provides the most accuracy in predictions. Figure 3 shows a comparison of the Akaike's information criterion (AIC) for the Gompertz model, Logistic model, and Baranyi model. The AIC is a measure of the goodness-of-fit of an estimated statistical model (18) and in most cases better model leads to a lower AIC value. It depends on the number of observed values, the sum of squares (SS), and the number of parameters in the model which is a very important standard for model selection. Consequently, the Gompertz model should be chosen to describe the growth of *S. aureus* in that the AIC values obtained from the Gompertz model at different temperatures are the lowest of all the other models.

Secondary modeling of *S. aureus* The Gompertz model showed the best statistical fit with the growth data of *S. aureus* in sandwich fillings at different temperatures compared with the Logistic model and the Baranyi model. Therefore, the SGR and LT calculated from the Gompertz formula analyzed by nonlinear regression were used to establish the secondary models to express the influence of storage temperature on the growth of *S. aureus* in sandwich fillings. The square root equation and natural logarithm equation constructed for SGR and LT, respectively, are exhibited in Table 1. Both the observed values and predicted values obtained from newly developed models are shown in Fig. 4 which indicates a good agreement between the observations and predictions with neglectable deviation. Further, graphical comparisons were carried out using a scatter plot to illustrate the fit of the proposed predicted model by plotting the observed SGR and LT against the predicted values. Figure 5 shows the observed values versus the predicted values, together with coefficient of determination (R^2) of 0.9901 and 0.9894, for SGR and LT, respectively. This indicates that both of the 2 models

Table 1. Mathematical validation factors calculated for secondary models developed for specific growth rate (SGR) and lag time (LT)

Equation	Indices[1)]	
	B_f	A_f
$SGR=(0.089T-0.117)^2$	0.993	1.156
$LT=e^{(-0.244T+3.503)}$	0.635	1.592

[1)] B_f, bias factor; A_f, accuracy factor

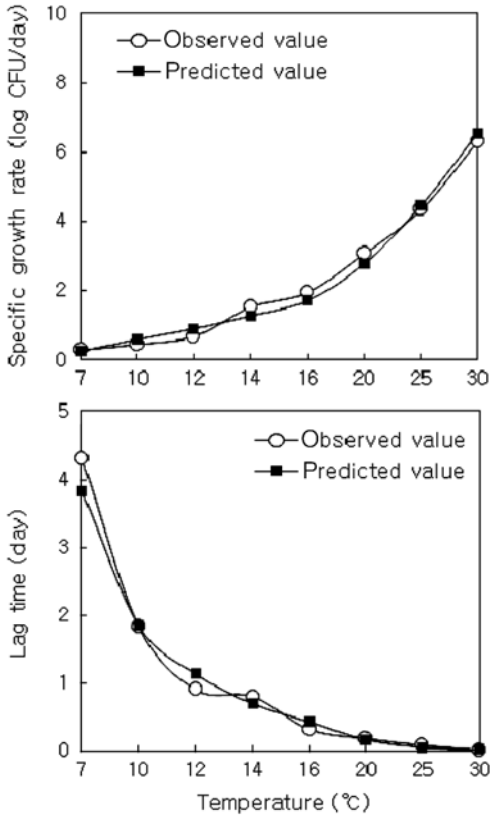

Fig. 4. Specific growth rate and lag time of *S. aureus* in sandwich fillings obtained from the predictive models.

can describe the growth data of *S. aureus* in sandwich fillings adequately.

Model evaluation Model evaluation is a necessary approach to evaluate the capability of a newly developed model to interpolate and it is a critical step in their development (19). The first stage is internal evaluation, which validates the model against the same data used for building the model (20-22). This ensures that the model accurately describes the data from which it was generated and represents any biological trend in those data (10). In this study, bias factor (B_f) and accuracy factor (A_f) are employed to assess the performance of presented models (Table 1), which are regarded as objective indications of model performance (22,23).

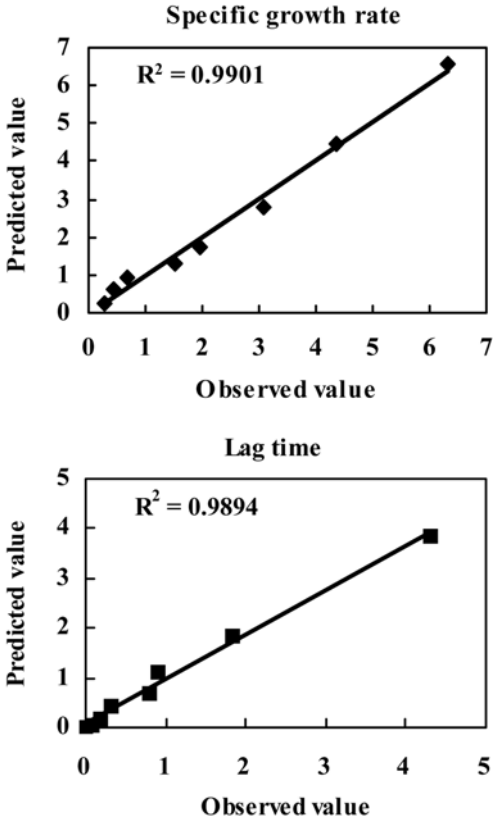

Fig. 5. Observed values vs. predicted values.

B_f is a measure of the extent of under- or over-prediction by the model of the SGR or LT observed (24). Generally, a B_f value of less than 1 indicates that the predicted value is higher than the observed value, which means fail safe. Similarly, a B_f with a value of more than 1 means fail dangerous indicating that the prediction resulted in a lower level of confidence and is lower than that of the observed value, which is also dangerous and needs to be avoided. This is an important parameter for the model under consideration (23). As Table 1 shows, the B_f values for the models developed for SGR and LT are 0.993 and 0.635, which denote the results are unsafe. Some previous published research also shows unsafe results. For example, Grau and Vanderlinde (17), Jin *et al.* (23), and Patterson *et al.* (25) reported fail-safe predictions on the B_f for a model developed for *L. monocytogenes*, and Baert *et al.* (26) published B_f values of 0.64-0.81 of SGR models describing the effect of storage temperature on the growth rate and lag phase of 6 *Penicillium expansum* strains. Until now, several standards for B_f values have been proposed. Armas *et al.* (27) considered that B_f values in the range 0.6-3.99 were acceptable for the specific growth rate of pathogens and spoilage organisms and Dalgaard and Jorgensen (19) suggested that B_f values within the range of 0.75-1.25 should be considered successfully validated for modeling of seafood spoilage microorganisms. Moreover, Ross (28) recommended the following interpretation of B_f when used for model performance evaluations involving pathogens: 0.90-1.05 could be considered as good; 0.70-0.90 or 1.06-1.15 can be considered as acceptable; less than 0.70 or greater than 1.15 should be considered as unacceptable. The different results might be dependent on the type of primary or secondary predictive models, type of samples used and various environmental factors.

In respect that B_f cannot provide the indication of the average accuracy of estimates because under- and over-prediction tend to cancel out (22). Therefore, the accuracy factor should be calculated since it averages the distance between each point and the line of equivalence as a measure of how close, on average, predictions are to the observations (29). Generally, the model was considered of high performance and accuracy if the value of A_f is 1, and a larger value of A_f results in lower accuracy of the average estimate. Table 1 shows the A_f values calculated for SGR and LT in this study are 1.156 and 1.592, respectively. There is no acknowledged standard for accuracy factor until now. The only publication which advised that an acceptable accuracy factor could be dependent on the number of environmental parameters in a kinetic model was reported by Ross *et al.* (30). In some previous reports, Jin *et al.* (23) found the A_f values calculated from SGR and LT models for *L. monocytogenes* in broth were 1.67 and 1.72, while Ding *et al.* (14) published a A_f value of 1.11 for SGR model of *L. monocytogenes* in lettuce treated with electrolyzed oxidizing water. Compared with the mentioned references or other publications (19,29,31), the A_f of our models seems good for validation.

In conclusion, 3 primary models were used to fit with the growth data of *S. aureus* in sandwich collected at various isothermal conditions. The Gompertz model provided the best statistic fit for growth data, compared with the Logistic model and Baranyi model. Consequently, secondary models were established for the specific growth rate (SGR) and lag time (LT) derived from the Gompertz model as a function of temperature. After evaluation of the developed models, the results indicated that the predicted values showed a good agreement with the observed ones, which demonstrated that the models developed in this study, can describe the experimental data well. Therefore, the newly constructed predictive models can be considered to provide a reliable prediction of *S. aureus* in sandwiches for sandwich manufacturers and HACCP programs.

Acknowledgments This work was supported by a research grant from the Korea Food & Drug Administration.

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