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Growth Characteristics of *Enterobacter sakazakii* Used to Develop a Predictive Model

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Abstract A mathematical model was developed for predicting the growth rate of *Enterobacter sakazakii* in tryptic soy broth medium as a function of the combined effects of temperature (5, 10, 20, 30, and 40°C), pH (4, 5, 6, 7, 8, 9, and 10), and the NaCl concentration (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10%). With all experimental variables, the primary models showed a good fit (R^2=0.8965 to 0.9994) to a modified Gompertz equation to obtain growth rates. The secondary model was 'ln specific growth rate= –0.38116+(0.01281*Temp)+(0.07993*pH)+(0.00618*NaCl)+(-0.00018*Temp^2)+(-0.00551*pH^2)+(-0.00093*NaCl^2) +(0.00013*Temp*pH)+(-0.00038*Temp*NaCl)+(-0.00023*pH*NaCl)'. This model is thought to be appropriate for predicting growth rates on the basis of a correlation coefficient (r) 0.9579, a coefficient of determination (R^2) 0.91, a mean square error 0.026, a bias factor 1.03, and an accuracy factor 1.13. Our secondary model provided reliable predictions of growth rates for *E. sakazakii* in broth with the combined effects of temperature, NaCl concentration, and pH.

Keywords: *Enterobacter sakazakii*, predictive model, growth rate

Introduction

Enterobacter sakazakii is a member of the family Enterobacteriaceae, genus *Enterobacter*. It is a motile, peritrichous, and Gram-negative rod (1). This organism was previously referred to as a 'yellow pigmented *Enterobacter cloacae*' (2). The first reported account of the bacterium now known as *E. sakazakii* was provided by the Japanese microbiologist Riichi Sakazaki (3) and the first description of the name *E. sakazakii* was by Farmer *et al.* (4) and Brenner *et al.* (5).

E. sakazakii has been associated most frequently with illness in neonates and children from 3 days to 4 years of age (6). The US Centers for Disease Control and Prevention reported an investigation into a 2001 Tennessee outbreak of *E. sakazakii* in a neonatal intensive care unit in which 10 cases were identified (7). *E. sakazakii* produces circulating microbial products, such as cell wall glycopeptides, endotoxins, proteases, collagenases, and elastases. And these products have been shown to induce permeability of the blood/brain barrier. In order to cause meningitis an organism has to colonize mucosal surfaces, translocate into the bloodstream, avoid host defense mechanisms, cross the blood/brain barrier, and survive in the cerebral spinal fluid (8). *E. sakazakii* infection induces fever, tachycardia, a decrease in vascular perfusions, and suspected seizure activity. As there is no epidemiological evidence on which to base a value for an *E. sakazakii* infectious dose, but it is reasonable to use 1,000 *E. sakazakii* cells as a first approximation. This is similar to the infectious dose of the pathogenic bacteria *Escherichia coli* O157 (9).

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Control of an *E. sakazakii* infection, like microbial food borne 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 environmental conditions, mathematical models provide a useful tool for predicting microbial growth (10). Impressive progress is being made in predictive microbiology and models are increasingly becoming standard research tools that are helpful in the evaluation and design of food-processing procedures. Use of predictive models may eventually become highly effective in the prevention of food borne outbreaks (11). Models, as a function of environmental control factors, will improve the shelf-life and safety of foods (12-16). However, this kind of growth predictive model was not yet been developed for *E. sakazakii*. All currently developed predictive models for *E. sakazakii* are related to thermal death and survival.

The objective of this study was to investigate the combined effects of temperature, NaCl concentration, and pH on the growth kinetics of *E. sakazakii* in a broth system. The goal was to develop a model that could be used to predict the maximum growth rates of the organism in any combination of the variables.

Materials and Methods

Bacterial culture *Enterobacter sakazakii* KCTC 2949 was used in the study. Tryptic soy broth (TSB, Difco Laboratories, Detroit, MI, USA) was used for maintenance and growth of the bacterial strain.

Experimental design A central composite design was used by incorporating variables and levels resulting in testing of 385 factor combinations. A polynomial model was developed for predicting the growth rate of *E.*

sakazakii in TSB medium as a function of the combined effects of temperature, pH, and NaCl concentration. TSB containing 11 different concentrations of NaCl (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10%, w/v) was adjusted to 7 different initial pH levels (pH 4, 5, 6, 7, 8, 9, and 10), with incubation at 5, 10, 20, 30, and 40°C.

Preparation and inoculation of culture media TSB containing 11 different concentrations of NaCl was autoclaved at 121°C for 15 min, and then allowed to cool. The pH of the media was then adjusted to an initial value of 4, 5, 6, 7, 8, 9, or 10 using 1 N NaOH or a 1 N HCl solution. Microplate wells were filled with 150 μL of each conditioned medium, to which 50 μL of inoculum containing 10^4 CFU/mL of *E. sakazakii* was added. Control wells containing 200 μL of un-inoculated medium were used as blanks and also used to check the sterility of the medium.

Growth temperature and growth rate measurement Growth rates of *E. sakazakii* in microplate wells incubated at 5, 10, 20, 30, and 40°C were measured every hour as the optical density at 600 nm by an automated microplate reader (ELx808; Biotech Ltd., Winooski, VI, USA). The observance values were natural log-transformed to homogenize variances.

Primary modeling Growth curves of the resulting absorbance vs. time values were iteratively generated using the modified Gompertz equation and fit to a nonlinear regression model (Prism version 4.0; GraphPad Software, San Diego, CA, USA) to determine maximum growth rates (GR, in log CFU/hr) at each incubation temperature.

$$Y=N_0+C*\exp[\exp\{(2.718*mue/C)*(Lag-X)+1\}]$$

The Gompertz parameter values were log cell number (Y), incubation time (X), and log initial number of cells (N_0). Measured values included the difference between the initial and final cell numbers (C), the lag time before growth (Lag), and the maximum specific growth rates (mue), as described by Gibson *et al.* (17).

Secondary modeling A polynomial model based on temperature, NaCl concentration, and pH was calculated for the growth rates. The Gompertz parameters for *E. sakazakii* growth data were determined using the least squares analysis (PROC GLM) of SAS version 8.1 (18).

$$\text{Ln growth rate}= b_0+b_1A+b_2B+b_3C+b_4A^2+b_5B^2+b_6C^2+b_7AB+b_8AC+b_9BC+\varepsilon$$

The model parameter values were incubation temperature (A), initial pH (B), NaCl concentration (C), regression coefficient (b_0-b_9), and random error (ε), as described by Gibson *et al.* (17).

Evaluation of model performance The correlation coefficient (r) and determination coefficient (R^2) (19) are often used as overall measures of a prediction. These values measure 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 that is not accounted for by deliberate changes in factors, such as temperature, pH, and Aw.

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

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

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

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 observations.

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

Results and Discussion

Primary modeling An absorbance measurement is regarded as an alternative method of measuring the viable count because the traditional viable count measurement for collecting growth data is time-consuming and labor intensive. However, the absorbance measurement is typically used to determine only growth rates (20-24) due to a high detection level that makes it difficult to measure the lag time (22). Therefore, the absorbance measurement was used for the development of a predictive growth model with growth rates of *E. sakazakii* in broth in this study.

Four main factors such as time, temperature, pH, and Aw were selected to measure growth characteristics of *E. sakazakii*. The growth rates of *E. sakazakii* at 7 different broth pH levels in the presence of 11 different concentrations of NaCl at incubation temperatures of 5, 10, 20, 30, and 40°C are shown in Table 1-4. No growth was observed in any combination of experimental variables at 5°C, NaCl 10%, or pH 4. At 10°C, growth of *E. sakazakii* was not observed at 4% NaCl at pH 10, 5% NaCl at pH 5, 6% NaCl at pH 6, 8, 9, 10, 7% NaCl at pH 4, 5, 8, 9, 10, and 8-10% NaCl at any pH. At 20°C, 3, 5, and 6% NaCl at pH 10, 8% NaCl at pH 5, 9, 10, and 9% NaCl showed no growth. At 30°C, less than 6% NaCl at pH 10, 7% NaCl at pH 9 and 10, 9% NaCl at pH 9 and 10, and 10% NaCl showed no growth. At 40°C, less than 6% NaCl at pH 10, 7% NaCl at pH 5, 9 and 10, 8% NaCl at pH 5, 8, 9 and 10, and over 9% NaCl showed no growth. The model developed involved 175 growth curves from 175 combinations of temperature, NaCl concentration, and pH in the broth.

The Gompertz equation is typically used by the U.S. Department of Agriculture to fit bacterial growth curves for estimation of lag time and maximum growth rates (25-28), so we used the Gompertz equation to fit growth curves for *E. sakazakii*. Best-fit values of growth rates for 175 growth curves in the primary model are shown in Table 1-4. Growth rate data for the broth showed a good fit for the Gompertz equation model with a high degree of goodness of fit ($R^2 = 0.8965$ to 0.9994) for all treatment factors (Table 1-4).

Secondary modeling The model we developed involved 175 growth curves for fewer than 385 combinations of temperature, NaCl concentration, and pH. The growth curves were transformed to natural logarithms to stabilize

Table 1. Best-fit growth rates (GR) of *Enterobacter sakazakii* in broth incubated at 10°C for primary modeling[1)]

T (°C)	NaCl (%)	pH	GR (1/hr)	R^2	T (°C)	NaCl (%)	pH	GR (1/hr)	R^2
10	0	4	NG	NA	10	3	4	NG	NA
10	0	5	0.0072	0.9890	10	3	5	0.0036	0.9302
10	0	6	0.0150	0.9867	10	3	6	0.0056	0.9137
10	0	7	0.0169	0.9650	10	3	7	0.0099	0.9067
10	0	8	0.0132	0.9742	10	3	8	0.0055	0.9877
10	0	9	0.0093	0.9489	10	3	9	0.0014	0.9103
10	0	10	0.0078	0.9074	10	3	10	0.0048	0.9412
10	1	4	NG	NA	10	4	4	NG	NA
10	1	5	0.0052	0.9580	10	4	5	0.0026	0.9769
10	1	6	0.0082	0.9930	10	4	6	0.0048	0.9142
10	1	7	0.0115	0.9894	10	4	7	0.0057	0.9492
10	1	8	0.0095	0.9225	10	4	8	0.0045	0.9119
10	1	9	0.0079	0.9254	10	4	9	0.0041	0.9253
10	1	10	0.0100	0.9330	10	4	10	NG	NA
10	2	4	NG	NA	10	5	4	NG	NA
10	2	5	0.0039	0.9902	10	5	5	NG	NA
10	2	6	0.0053	0.9339	10	5	6	0.0032	0.9352
10	2	7	0.0096	0.9373	10	5	7	0.0052	0.9500
10	2	8	0.0079	0.9892	10	5	8	0.0041	0.9249
10	2	9	0.0063	0.9003	10	5	9	0.0043	0.9356
10	2	10	NG	NA	10	5	10	0.0024	0.9209
10	6	4	NG	NA	10	9	4	NG	NA
10	6	5	NG	NA	10	9	5	NG	NA
10	6	6	0.0055	0.9237	10	9	6	NG	NA
10	6	7	0.0021	0.9331	10	9	7	NG	NA
10	6	8	NG	NA	10	9	8	NG	NA
10	6	9	NG	NA	10	9	9	NG	NA
10	6	10	NG	NA	10	9	10	NG	NA
10	7	4	NG	NA	10	10	4	NG	NA
10	7	5	NG	NA	10	10	5	NG	NA
10	7	6	0.0019	0.9389	10	10	6	NG	NA
10	7	7	0.0022	0.9522	10	10	7	NG	NA
10	7	8	NG	NA	10	10	8	NG	NA
10	7	9	NG	NA	10	10	9	NG	NA
10	7	10	NG	NA	10	10	10	NG	NA
10	8	4	NG	NA					
10	8	5	NG	NA					
10	8	6	NG	NA					
10	8	7	NG	NA					
10	8	8	NG	NA					
10	8	9	NG	NA					
10	8	10	NG	NA					

[1)]GR, growth rate; R^2, coefficient of determination; NG, no growth; NA, no application.

model variance (17) and were subjected to polynomial analysis using the SAS general linear model. The following equation was determined:

$$\begin{aligned}\text{Ln growth rate} = &-0.38116+(0.01281*\text{Temp})+(0.07993*\text{pH})\\ &+(0.00618*\text{NaCl})+(-0.00018*\text{Temp}^2)\\ &+(-0.00551*\text{pH}^2)+(-0.00093*\text{NaCl}^2)\\ &+(0.00013*\text{Temp}*\text{pH})+(-0.00038*\text{Temp}*\text{NaCl})\\ &+(-0.00023*\text{pH}*\text{NaCl})\end{aligned}$$

This equation is an appropriate secondary model for growth rate on the basis of the correlation coefficient ($r=0.9579$) and the determination coefficient ($R^2=0.91$) for estimation of the predicted growth rates of *E. sakazakii* in different combinations of temperature, NaCl concentration, and pH in broth, as shown in Fig. 1-4. When the overall main effects of NaCl concentration and pH level in broth incubated at 10, 20, 30, and 40°C were compared, the predicted growth rates of *E. sakazakii* were generally decreased by both basic (pH 9-10) and acidic (pH 5-6) conditions and higher NaCl concentrations (Fig. 1-4).

Table 2. Best-fit growth rates (GR) of *Enterobacter sakazakii* in broth incubated at 20°C for primary modeling[1)]

T (°C)	NaCl (%)	pH	GR (1/hr)	R^2	T (°C)	NaCl (%)	pH	GR (1/hr)	R^2
20	0	4	NG	NA	20	3	4	NG	NA
20	0	5	0.0997	0.9876	20	3	5	0.0693	0.9720
20	0	6	0.1093	0.9915	20	3	6	0.0907	0.9616
20	0	7	0.1181	0.9868	20	3	7	0.1021	0.9504
20	0	8	0.1148	0.9918	20	3	8	0.0908	0.9500
20	0	9	0.1074	0.9594	20	3	9	0.0870	0.9052
20	0	10	0.0838	0.9204	20	3	10	NG	NA
20	1	4	NG	NA	20	4	4	NG	NA
20	1	5	0.0916	0.9593	20	4	5	0.0371	0.9925
20	1	6	0.1040	0.9852	20	4	6	0.0451	0.9748
20	1	7	0.1023	0.9746	20	4	7	0.0601	0.9615
20	1	8	0.1094	0.9621	20	4	8	0.0552	0.9641
20	1	9	0.1038	0.9198	20	4	9	0.0468	0.9747
20	1	10	0.0526	0.9866	20	4	10	0.0399	0.9521
20	2	4	NG	NA	20	5	4	NG	NA
20	2	5	0.0882	0.9617	20	5	5	0.0362	0.9766
20	2	6	0.0968	0.9519	20	5	6	0.0458	0.9925
20	2	7	0.1057	0.9653	20	5	7	0.0542	0.9940
20	2	8	0.0987	0.9660	20	5	8	0.0513	0.9763
20	2	9	0.0957	0.9533	20	5	9	0.0434	0.9293
20	2	10	0.0957	0.9615	20	5	10	NG	NA
20	6	4	NG	NA	20	9	4	NG	NA
20	6	5	0.0357	0.9715	20	9	5	NG	NA
20	6	6	0.0389	0.9751	20	9	6	NG	NA
20	6	7	0.0455	0.9850	20	9	7	NG	NA
20	6	8	0.0425	0.9759	20	9	8	NG	NA
20	6	9	0.0399	0.9626	20	9	9	NG	NA
20	6	10	NG	NA	20	9	10	NG	NA
20	7	4	NG	NA	20	10	4	NG	NA
20	7	5	0.0284	0.9936	20	10	5	NG	NA
20	7	6	0.0370	0.9913	20	10	6	NG	NA
20	7	7	0.0412	0.9928	20	10	7	NG	NA
20	7	8	0.0379	0.9792	20	10	8	NG	NA
20	7	9	0.0289	0.9547	20	10	9	NG	NA
20	7	10	0.0318	0.9437	20	10	10	NG	NA
20	8	4	NG	NA	20				
20	8	5	NG	NA	20				
20	8	6	0.0308	0.9872	20				
20	8	7	0.0378	0.9948	20				
20	8	8	0.0372	0.9948	20				
20	8	9	NG	NA	20				
20	8	10	NG	NA	20				

[1)]GR, growth rate; R^2, coefficient of determination; NG, no growth; NA, no application.

However, the predicted growth rates of *E. sakazakii* were not inhibited more at pH 8 than at pH 7 at all temperatures. 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 for storage at 10°C than at 20, 30, and 40°C. Therefore, we compared our results with the growth of predictive models of other microorganisms, including *Salmonella enterica* serovar Typhimurium (*Salmonella* Typhimurium), *Listeria monocytogenes, Staphylococcus aureus* (29-31). These 3 bacteria, main food-borne pathogens worldwide are facultative anaerobes, live in animal GI tract and possess similar physiological properties. Table 6 shows growth predictive model comparisons for *E. sakazakii,* and 3 other microorganisms. Growth rates were calculated from each predictive model. The predictive growth rate of *E. sakazakii* is the lowest.

Evaluation of model performance Table 5 presents 4

Table 3. Best-fit growth rates (GR) of *Enterobacter sakazakii* in broth incubated at 30°C for primary modeling[1)]

T (°C)	NaCl (%)	pH	GR (1/hr)	R^2	T (°C)	NaCl (%)	pH	GR (1/hr)	R^2
30	0	4	NG	NA	30	3	4	NG	NA
30	0	5	0.1587	0.9893	30	3	5	0.0676	0.9979
30	0	6	0.1633	0.9862	30	3	6	0.1406	0.9648
30	0	7	0.1663	0.9698	30	3	7	0.1498	0.9158
30	0	8	0.1627	0.9850	30	3	8	0.1404	0.9151
30	0	9	0.1595	0.9848	30	3	9	0.1276	0.9528
30	0	10	NG	NA	30	3	10	NG	NA
30	1	4	NG	NA	30	4	4	NG	NA
30	1	5	0.0854	0.9859	30	4	5	0.0607	0.9334
30	1	6	0.1505	0.9757	30	4	6	0.1149	0.9863
30	1	7	0.1591	0.9664	30	4	7	0.1340	0.9807
30	1	8	0.1516	0.9386	30	4	8	0.1283	0.9745
30	1	9	0.1486	0.9465	30	4	9	0.1102	0.9453
30	1	10	NG	NA	30	4	10	0.1102	0.9040
30	2	4	NG	NA	30	5	4	NG	NA
30	2	5	0.0794	0.9911	30	5	5	0.0594	0.9628
30	2	6	0.1335	0.9732	30	5	6	0.0956	0.9886
30	2	7	0.1518	0.9845	30	5	7	0.1034	0.9760
30	2	8	0.1440	0.9555	30	5	8	0.0990	0.9725
30	2	9	0.1345	0.9747	30	5	9	0.0913	0.9114
30	2	10	0.1261	0.9050	30	5	10	NG	NA
30	6	4	NG	NA	30	9	4	NG	NA
30	6	5	0.0562	0.9422	30	9	5	0.0076	0.9869
30	6	6	0.0921	0.9864	30	9	6	0.0101	0.9864
30	6	7	0.0973	0.9802	30	9	7	0.0086	0.9352
30	6	8	0.0964	0.9802	30	9	8	0.0141	0.9919
30	6	9	0.0896	0.9306	30	9	9	NG	NA
30	6	10	NG	NA	30	9	10	NG	NA
30	7	4	NG	NA	30	10	4	NG	NA
30	7	5	0.0415	0.9837	30	10	5	NG	NA
30	7	6	0.0623	0.9803	30	10	6	NG	NA
30	7	7	0.0728	0.9767	30	10	7	NG	NA
30	7	8	0.0681	0.9897	30	10	8	NG	NA
30	7	9	NG	NA	30	10	9	NG	NA
30	7	10	NG	NA	30	10	10	NG	NA
30	8	4	NG	NA	30				
30	8	5	NG	NA	30				
30	8	6	0.0342	0.9879	30				
30	8	7	0.0518	0.9653	30				
30	8	8	0.0203	0.9890	30				
30	8	9	NG	NA	30				
30	8	10	NG	NA	30				

[1)]GR, growth rate; R^2, coefficient of determination; NG, no growth; NA, no application.

different statistical indices of the secondary modeling step for the predicted growth rates of *E. sakazakii* in broth. Higher values of r and R^2 result in better prediction by the model (32-34) and a lower value of MSE results in better adequacy of the model to describe the data (34, 35). $B_f<1$ indicates a 'fail safe' model and $B_f>1$ indicates a 'fail dangerous' model (36). Ross (37) also noted, for models describing pathogen growth rates, that B_f in the range 0.9-1.05 can be considered good, in the range 0.7-0.9 or 1.06-1.15 considered acceptable, and <0.7 or >1.5 considered unacceptable. A larger value of A_f results in lower accuracy of the average estimate. An acceptable model that predicts the growth rates of *S.* Typhimurium and *L. monocytogenes* as a function of temperature, NaCl concentration, and pH is expected to have an A_f value in the range 1.3-1.5 (29-31). When $A_f=B_f=1$, the predictive model is perfect.

Our results indicate that the developed polynomial model provides reliable predictions for growth rates of *E. sakazakii* in broth under different combinations of temperature, NaCl concentration, and pH. However, for

Table 4. Best-fit growth rates (GR) of *Enterobacter sakazakii* in broth incubated at 40°C for primary modeling[1)]

T (°C)	NaCl (%)	pH	GR (1/hr)	R^2	T (°C)	NaCl (%)	pH	GR (1/hr)	R^2
40	0	4	NG	NA	40	3	4	NG	NA
40	0	5	0.085	0.999	40	3	5	0.083	0.996
40	0	6	0.172	0.994	40	3	6	0.134	0.988
40	0	7	0.186	0.978	40	3	7	0.144	0.976
40	0	8	0.181	0.975	40	3	8	0.135	0.909
40	0	9	0.151	0.983	40	3	9	0.130	0.903
40	0	10	0.120	0.903	40	3	10	NG	NA
40	1	4	NG	NA	40	4	4	NG	NA
40	1	5	0.098	0.983	40	4	5	0.078	0.957
40	1	6	0.144	0.989	40	4	6	0.127	0.984
40	1	7	0.160	0.980	40	4	7	0.132	0.943
40	1	8	0.153	0.935	40	4	8	0.125	0.917
40	1	9	0.143	0.913	40	4	9	0.122	0.911
40	1	10	0.103	0.907	40	4	10	0.105	0.902
40	2	4	NG	NA	40	5	4	NG	NA
40	2	5	0.095	0.996	40	5	5	0.052	0.998
40	2	6	0.137	0.988	40	5	6	0.081	0.987
40	2	7	0.154	0.924	40	5	7	0.123	0.985
40	2	8	0.148	0.902	40	5	8	0.116	0.949
40	2	9	0.141	0.900	40	5	9	0.080	0.900
40	2	10	NG	NA	40	5	10	NG	NA
40	6	4	NG	NA	40	9	4	NG	NA
40	6	5	0.044	0.937	40	9	5	NG	NA
40	6	6	0.078	0.984	40	9	6	NG	NA
40	6	7	0.111	0.970	40	9	7	NG	NA
40	6	8	0.106	0.934	40	9	8	NG	NA
40	6	9	0.090	0.897	40	9	9	NG	NA
40	6	10	NG	NA	40	9	10	NG	NA
40	7	4	NG	NA	40	10	4	NG	NA
40	7	5	NG	NA	40	10	5	NG	NA
40	7	6	0.020	0.984	40	10	6	NG	NA
40	7	7	0.023	0.995	40	10	7	NG	NA
40	7	8	0.016	0.945	40	10	8	NG	NA
40	7	9	NG	NA	40	10	9	NG	NA
40	7	10	NG	NA	40	10	10	NG	NA
40	8	4	NG	NA	40				
40	8	5	NG	NA	40				
40	8	6	0.012	0.988	40				
40	8	7	0.017	0.921	40				
40	8	8	NG	NA	40				
40	8	9	NG	NA	40				
40	8	10	NG	NA	40				

[1)]GR, growth rate; R^2, coefficient of determination; NG, no growth; NA, no application.

Table 5. Statistical indices for the secondary response surface modeling step for growth rates of *Enterobacter sakazakii* in broth[1)]

Indicator	r	R^2	MSE	B_f	A_f
Response surface model	0.9579	0.9176	0.0258	1.0300	1.1308

[1)]r, Correlation coefficient (99% confidence interval); R^2, coefficient of determination (99% confidence interval); MSE, mean square error; B_f, bias factor; A_f, accuracy factor.

risk management, further work is necessary to confirm the predictions of this growth rate model for use with food products. There is an urgent necessity for development of models of growth, death, survival, and transmission of *E. sakazakii* in diverse food matrices and food processing plants exposed to various environmental conditions. Our

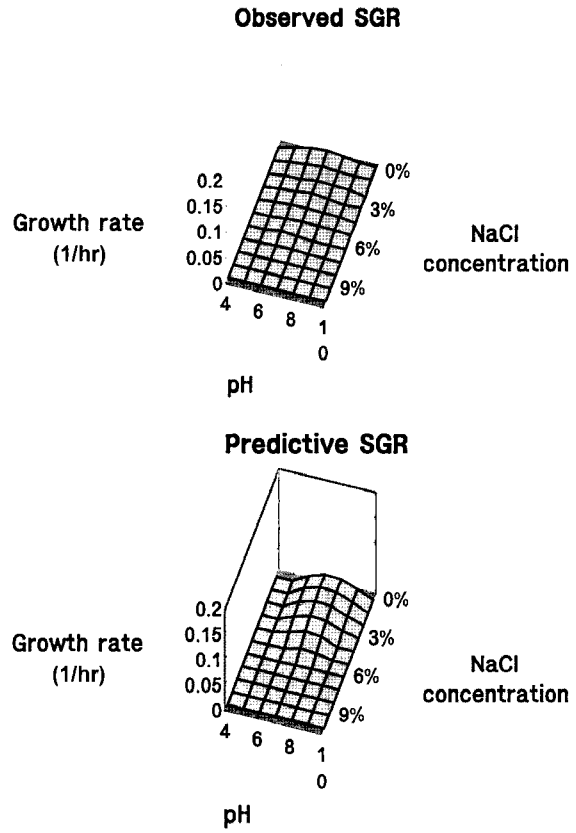

Fig. 1. Predicted and observed specific growth rates (SGR) of *Enterobacter sakazakii* in broth incubated at 10°C with various concentrations of NaCl and different pH values.

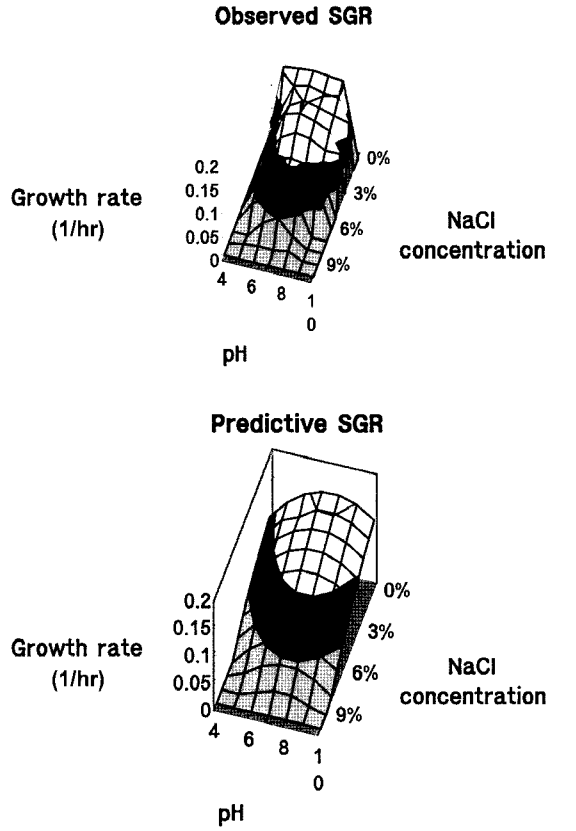

Fig. 3. Predicted and observed specific growth rates (SGR) of *Enterobacter sakazakii* in broth incubated at 30°C with various concentrations of NaCl and different pH values.

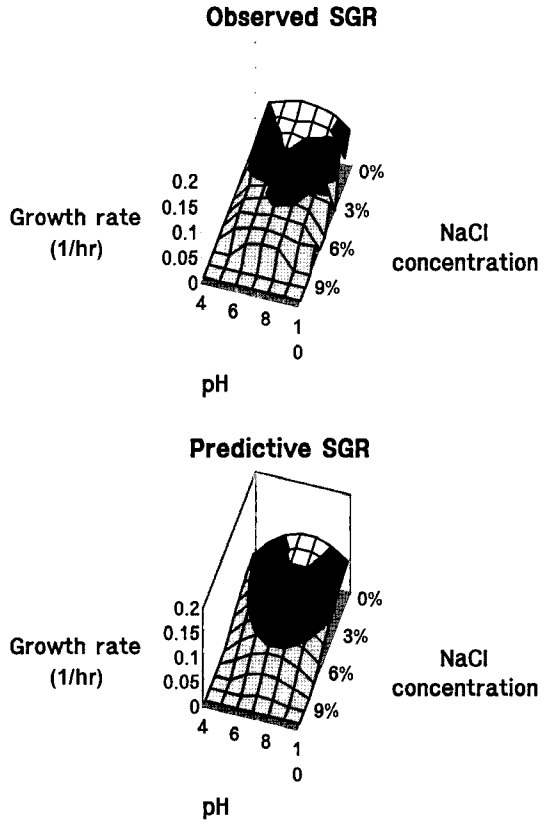

Fig. 2. Predicted and observed specific growth rates (SGR) of *Enterobacter sakazakii* in broth incubated at 20°C with various concentrations of NaCl and different pH values.

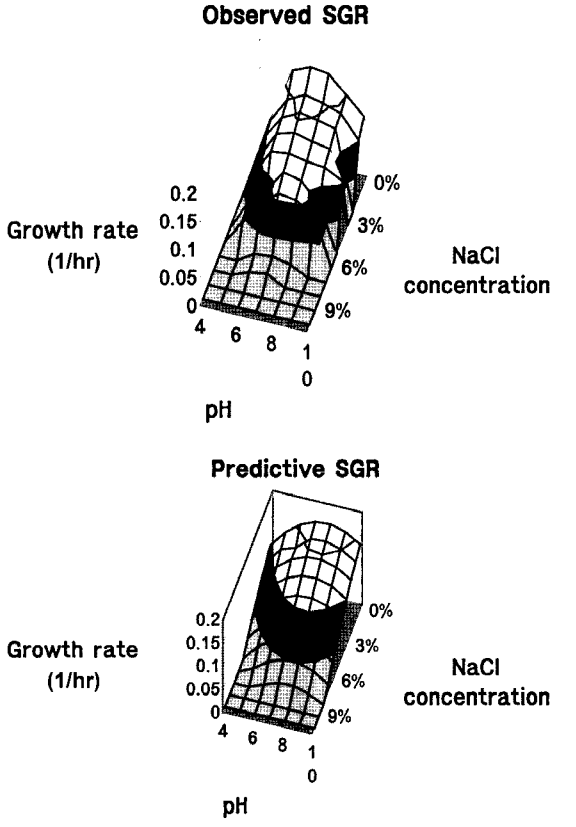

Fig. 4. Predicted and observed specific growth rates (SGR) of *Enterobacter sakazakii* in broth incubated at 40°C with various concentrations of NaCl and different pH values.

Table 6. Maximum and average predictive growth rates for microorganisms based on predictive models

Growth rate	Bacteria	10°C	20°C	30°C	40°C
Maximum growth rate (1/hr)	*Listeria monocytogenes*	0.055	0.219	0.488	0.600
	Staphylococcus aureus	0.057	0.180	0.330	0.494
	Salmonella Typhimurium	0.051	0.129	0.209	0.291
	Enterobacter sakazakii	0.028	0.111	0.107	0.159
Average growth rate (1/hr)[1)]	*Listeria monocytogenes*	0.028	0.114	0.258	0.320
	Staphylococcus aureus	0.026	0.108	0.217	0.356
	Salmonella Typhimurium	0.028	0.074	0.118	0.174
	Enterobacter sakazakii	0.016	0.089	0.065	0.081

[1)]Average growth rate; all NaCl concentrations (0, 2, 4, 6, 8, and 10%) and all pH values (4, 5, 6, 7, 8, 9, and 10) for predictive growth rates used to calculate an average.

developed growth rate model can be used to reduce the risk of *E. sakazakii* in food processing and distribution to ensure food safety (38).

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