J. Microbiol. Biotechnol. (2009), **19**(7), 718–726
doi: 10.4014/jmb.0809.534
First published online 24 February 2009

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Development and Validation of a Predictive Model for *Listeria monocytogenes* Scott A as a Function of Temperature, pH, and Commercial Mixture of Potassium Lactate and Sodium Diacetate

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Received: September 19, 2008 / Revised: January 5, 2009 / Accepted: January 8, 2009

The objective of this study was to develop and validate secondary models that can predict growth parameters of *L. monocytogenes* Scott A as a function of concentrations (0–3%) of a commercial potassium lactate (PL) and sodium diacetate (SDA) mixture, pH (5.5–7.0), and temperature (4–37°C). A total of 120 growth curves were fitted to the Baranyi primary model that directly estimates lag time (LT) and specific growth rate (SGR). The effects of the variables on *L. monocytogenes* Scott A growth kinetics were modeled by response surface analysis using quadratic and cubic polynomial models of the natural logarithm transformation of both LT and SGR. Model performance was evaluated with dependent data and independent data using the prediction bias (B_f) and accuracy factors (A_f) as well as the acceptable prediction zone method [percentage of relative errors (%RE)]. Comparison of predicted versus observed values of SGR indicated that the cubic model fits better than the quadratic model, particularly at 4 and 10°C. The B_f and A_f for independent SGR were 1.00 and 1.08 for the cubic model and 1.08 and 1.16 for the quadratic model, respectively. For cubic and quadratic models, the %REs for the independent SGR data were 92.6 and 85.7, respectively. Both quadratic and cubic polynomial models for SGR and LT provided acceptable predictions of *L. monocytogenes* Scott A growth in the matrix of conditions described in the present study. Model performance can be more accurately evaluated with B_f and A_f and % RE together.

Keywords: *L. monocytogenes* Scott A, polynomial model, model validation, potassium lactate/diacetate mixture, temperature

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Predictive growth modeling of *L. monocytogenes* has received a lot of attention [2, 9, 16] because of listeriosis outbreaks, predominantly associated with ready-to-eat (RTE) food. If models can be developed to give reliable predictions, considerable savings can be made in costs associated with laboratory challenge testing of food products. Furthermore, these models can be utilized by the food industry and risk assessors to control the safety and quality of food and to quantify the effects of environmental factors on the behavior of the pathogen.

An important step after developing a model is to evaluate the performance of the model by comparing its predictions against observed data. Performance evaluation can be carried out on the basis of the data used in model development to determine if the model sufficiently describes the experimental data (internal validation) [24]. External validation uses new data that were obtained from growth data reported in the literature. However, the problem with literature data is that the comparisons are often confounded by more than one experimental variable being different than the data used in model development. In addition, independent data that were not used in model development but were inside model boundaries (interpolation) can be used for internal validation [17]. The adequacy of the model to predict data should be assessed both graphically using plots of prediction errors as well as by using mathematical and/or statistical indices that quantify prediction bias and accuracy [4, 14]. Quantifying model performance using prediction bias (B_f) and accuracy factors (A_f) [19] is the widely used method in predictive microbiology. However, these performance indices have limitations, because B_f and A_f are based on average values, and prediction cases involving no growth are excluded from calculation of B_f and A_f, which can result in an

overestimation of model performance [2, 6]. In addition, when a model shows underprediction in one region of the response surface and overprediction in another region of the response surface, an acceptable B_f and A_f could be observed [19]. The acceptable prediction zone method has added another approach to evaluating model performance that overcomes the aforementioned limitations of B_f and A_f. The acceptable prediction zone method has a single performance factor, which is the percentage of relative errors (%RE) that fall in an acceptable prediction zone, which assesses both prediction bias and accuracy factor [17].

The recent interest in use of lactate and acetate salts is attributed to their potential to inhibit spoilage and pathogenic bacteria [10, 13, 23, 26, 27, 28]. They are commercially available in liquid (60% wt/wt) form and are widely used in processed meat formulations. Consequently, lactate and diacetate have been incorporated as a variable in the mathematical models to predict various products safety, including cooked meat products [7, 12], bologna-type sausages [9], beef [11], lightly preserved seafood [15], frankfurter slurry [21], and cured processed meat products [22]. In addition, a commercially used product known as PURASAL P Opti.Form 4, which is a combination of potassium lactate (PL) with sodium diacetate (SDA), was used effectively to control *L. monocytogenes* Scott A in broth [1] and in a cold smoked salmon model [26, 28].

In the present study, we developed secondary models that can predict growth parameters of *L. monocytogenes* Scott A as a function of concentrations (0–3%) of a PL and SDA mixture and storage conditions of pH (5.5–7.0) and temperature (4–37°C), which are major parameters affecting the growth kinetics of *L. monocytogenes* in ready-to-eat meat products in the retail market. This is the first set of static broth models reported in the literature where the interaction of potassium lactate–sodium diacetate concentration, pH, and temperature are detailed. In addition, the developed models were evaluated for model performance using the prediction bias (B_f) and accuracy factors (A_f) for both dependent and independent data as well as the acceptable prediction zone method (% RE).

Materials and Methods

Culture and Inoculum Preparation

The L. monocytogenes Scott A strain (ATCC 49594) was purchased from the American Type Culture Collection (Rockville, MD, U.S.A.). The strain was maintained at −70°C at a concentration of about 9.0 log CFU/ml in brain heart infusion broth (BHI) (Difco, Detroit, MI, U.S.A.) with 15% glycerol. Stock cultures were thawed at room temperature and then 10 µl was inoculated under aseptic conditions into a 25-ml Erlenmeyer flask containing 9 ml of sterile TSB–YE (trypticase soy broth with 0.6% yeast extract). The flask was sealed with a foam plug and incubated on a rotary shaker (150±2 rpm) for 24 h at 37°C under aerobic conditions. The pathogen culture was grown until the late exponential phase of growth (~8 log CFU/ml). Under aseptic conditions, 1 ml of the culture was serially diluted into 9 ml of 0.1% sterilized peptone water for inoculation into the BHI broths.

Potassium Lactate and Sodium Diacetate Mixture

The mixture of PL and SDA was obtained from PURAC America Inc. (Lincolnshire, IL, U.S.A.). The solution, which is commercially known as PURASAL P Opti.Form 4, was prepared from potassium L-2 hydroxypropionate and sodium hydrogen diacetate. PURASAL P Opti.Form 4 is a 60% HiPure grade solution with 56% PL and 4% SDA.

Broth Preparation and Inoculation Procedures

BHI broth was used as the culture medium for all experiments. A volume of 50 ml of BHI broth was prepared in five 250-ml Erlenmeyer flasks. One flask served as the control where no PURASAL P Opti. Form 4 was added. To the other four flasks, 0.3% (0.168 lactate–0.012 diacetate), 1.0% (0.56 lactate–0.04 diacetate), 1.8% (1.008 lactate–0.072 diacetate), or 3% (1.68 lactate–0.12 diacetate) of PURASAL P Opti. Form 4 was added. The pH values of the BHI were then adjusted to 5.5, 6.0, 6.5, and 7.0 using 1 M HCl or 1 M NaOH and measured using an IQ 240 pH meter with a non-glass probe (IQ Scientific Instruments, Inc., San Diego, CA, U.S.A.). All flasks were sealed with a foam plug and autoclaved at 121°C for 15 min and the pH of each BHI broth was readjusted, if necessary. To adjust the broth incubation temperature before inoculation, all flasks with BHI were kept overnight in the appropriate incubator after autoclaving. On the following day, each flask was aseptically inoculated with 50 µl of a diluted culture of *L. monocytogenes* Scott A to reach an initial population of 10^2 CFU/ml. In addition, higher concentrations of log CFU/ml between 1.8 and 4.1 log CFU/ml were also prepared using the same protocol to test the influence of inoculum size on specific growth rates (SGR), lag times (LT), and maximum population densities (MPD) of *L. monocytogenes* Scott A in BHI broth. Flasks were immediately incubated at 4, 10, or 17°C (Model 2005; Sheddon Manufacturing, Cornelius, OR, U.S.A.) or at 20, 24, 30, and 37°C (Model 4230; New Brunswick, Edison, NJ, U.S.A.).

Enumeration of *L. monocytogenes* Scott A

At intervals post inoculation and during storage, depending on the incubation temperature and pH of broth, 50 µl of two dilutions of the test BHI were spiral plated (Autoplate 4000; Spiral Biotech Inc., Norwood, MA, U.S.A.) onto tryptose agar. Plates were counted after 24 to 30 h incubation at 37°C. Colonies on triplicate plates of each sample were counted with an automated colony counter (Q Count; Spiral Biotech Inc. Norwood, MA, U.S.A.). The number of colonies expressed as $\log_{10}$ CFU/ml was converted to the natural log (ln) as dictated by use of the Baranyi primary model [3]. The mean number of the triplicate determinations per sample was used in primary modeling.

Experimental Design

A complete factorial design was used to assess the effects of temperature, pH, and concentrations of PL and SDA mixture. The boundaries of the mixture of PL and SDA concentrations were chosen after preliminary screening experiments to determine the growth limits at each pH and temperature. A total of 120 experimental combinations of temperature (4, 10, 17, 24, 30, 37°C), pH (5.5, 6.0,

6.5, 7.0), and concentrations of PL and SDA mixture (0.0, 0.3, 1.0, 1.8, 3.0%) were examined in three replicates. A minimum of 12 data points were analyzed for each curve; at least 3 points during the lag phase, 5 points during the exponential phase, and 3 points to define the stationary phase.

Primary Modeling

The mean number of the triplicate plate counts of *L. monocytogenes* Scott A was graphed as a function of time. The resulting growth curves were iteratively fit to the Baranyi model [3] using DMFit version 2.0, an Excel add-in for fitting sigmoid functions, to determine the specific growth rate (ln CFU/h) and lag time(h).

$$y(t)=y_o+\mu_{max}A(t)-\frac{1}{m}In\left(1+\frac{e^{m\mu_{max}A(t)}-1}{e^{m(y_{max}-y_0)}}\right) \quad (1)$$

$$A(t)=t+\frac{1}{v}In\left(\frac{e^{-vt}+q_o}{1+q_o}\right) \quad (2)$$

where y(t) = ln x(t), x(t) is the number of cells at time t, y_o=ln x_o, x_o is the number of cells at time t=0, q_o=the concentration of limiting substrate at t=0, μ_{max}=the specific growth rate, m=a curvature parameter to characterize the transition from exponential phase to stationary phase, and v=the rate of increase of the limiting substrate, generally assumed to be equal to μ_{max}.

Secondary Modeling

Quadratic and cubic response surface equations in terms of temperature, pH, and the mixture of PL and SDA concentrations were developed for specific growth rate (SGR:μ_{max}) and lag time (LT:λ) of *L. monocytogenes* Scott A in BHI broth by multiple regression using the SAS (v 8.02) General Linear Models Procedure:

$$\ln\ y=a_o+a_1A+a_2B+a_3C+a_4AB+a_5AC+a_6BC+a_7ABC....a_iA^n+a_iB^n+a_iC^n+e \quad (3)$$

where ln y is the natural logarithm of the modeled growth parameters (μ_{max} or λ) for the polynomial model, a_i (i=1, 2, 3…..) are coefficients to be estimated, A is the temperature, B is the pH, C is the mixture of PL and SDA concentration, and e is the random error assumed to have a zero mean and constant variance.

Model Performance

The models were first evaluated using the same data as were used to develop the model to determine if the model can describe the experimental data sufficiently; that is, internal validation [24] or goodness-of-fit. Next, the model performance for interpolation was evaluated using independent data sets for selected conditions, which were not used in model development. The conditions selected were within the response surface model boundaries (interpolation) that were expected to show growth rather than death rates, and the pHs selected were around that of meat products. Therefore, the experimental design for internal validation was a full 5×2×3 factorial arrangement of temperature (7, 14, 20, 27, 33°C), pH (5.7, 6.3), and levels of the mixture of PL and SDA (0.0, 0.5, 1.5%) in BHI. Data were collected with the same strain, growth media, and modeling procedures.

For dependent and independent data, prediction bias (B_f) and accuracy factors (A_f) were calculated using the following formula [19]:

$$B_f \text{ for LT}=10^{\Sigma\log(\text{predicted/observed})/n} \quad (4)$$

$$A_f \text{ for LT}=10^{(\Sigma|\log(\text{predicted/observed})|/n)} \quad (5)$$

$$B_f \text{ for SGR}=10^{\Sigma\log(\text{observed/predicted})/n} \quad (6)$$

$$A_f \text{ for SGR}=10^{(\Sigma|\log(\text{observed/predicted})|/n)} \quad (7)$$

where the mean values for B_f and A_f were used as overall measures of model prediction bias and accuracy, respectively. Different ratios were used for LT and SGR, so that B_f and A_f less than 1 would represent fail-safe predictions, and B_f and A_f above 1 would represent fail-dangerous predictions. Likewise, relative errors (RE) of individual prediction cases were calculated [6]:

$$\text{RE for SGR}=[(\text{observed}-\text{predicted})/\text{predicted}]\times100 \quad (8)$$

$$\text{RE for LT}=[(\text{predicted}-\text{observed})/\text{predicted}]\times100 \quad (9)$$

where RE less than zero represented fail-safe predictions and RE above zero represented fail-dangerous predictions.

In the acceptable prediction zone method for SGR, the percentage of RE (%RE) that is in an acceptable prediction zone (*i.e.*, number of RE in the acceptable prediction zone/total number of prediction cases) from −30% (fail-safe) to 15% (fail-dangerous) is calculated and used as a new measure of model performance. Models with %RE ≥70 are considered to provide predictions with acceptable bias and accuracy [17]. Because of differences between experimental errors associated with different kinetic parameters (SGR, LT, and MPD), different acceptable prediction zones are used for evaluating the performance of individual parameters [18]. Thus, the width of the acceptable prediction zone is most narrow for SGR (−30% to 15%), intermediate for LT (−60% to 30%), and widest for MPD models (−80% to 40%)[18].

RESULT AND DISCUSSION

Effect of Inoculum Size on Growth Kinetics

Initial experiments indicated that the growth kinetics of *L. monocytogenes* were not influenced by the initial population density (inoculum size) between 1.8 and 4.1 log CFU/ml. More specifically, these data indicated that initial population density did not affect (P>0.05) the specific growth rate, lag time, or maximum population density of *L. monocytogenes* in BHI broth incubated at 10 or 17°C (Table 1). This indicated that the initial contamination level of pathogens was not a major factor to affect the growth kinetics of pathogen in foods. The independence of MPD, LT, and SGR from the initial innoculum size under specific environmental conditions has been previously demonstrated by Buchanan and Phillips [5].

Modeling Growth of *L. monocytogenes* Scott A

Among the 120 combination treatments of temperature, pH, and potassium lactate and sodium diacetate mixture tested in the present study, growth of *Listeria monocytogenes* Scott A was observed in 99 treatments and no growth in 21 treatments. The growth curves were fit to the Baranyi primary model after transformation of CFU counts to natural logarithms (ln) [3].

In secondary modeling, LT and SGR values from the primary modeling were transformed to their natural logarithm

Table 1. Effect of initial population density (IPD) on specific growth rate (SGR), lag time (LT), and maximum population density (MPD) of *L. monocytogenes* Scott A.

Temperature (°C)	IPD[a] (log CFU/ml)	SGR (log CFU/h)	LT (h)	MPD (log CFU/ml)
10	1.8	0.027	154.11	8.88
10	2.2	0.029	149.51	8.81
10	3.7	0.026	163.28	8.89
10	4.1	0.027	159.27	8.79
17	1.8	0.083	27.40	8.58
17	2.2	0.079	26.14	8.59
17	3.7	0.084	25.98	8.52
17	4.1	0.085	28.18	8.62

[a]Triplicate flasks of broth with 1.0% potassium lactate/sodium diacetate mixture, pH 6.0.

to stabilize the model variance and regressed against model variables (*i.e.*, temperature, pH, and the concentration of PL and SDA mixture) [8]. Data and culture conditions supporting growth and no growth along with the corresponding SGR, LT, and MPD obtained from primary modeling are given in Table 2. At culture conditions that did not support growth, the CFU counts decreased from the initial population of about 2.5 log CFU/ml, and any of the experimental conditions in which death was observed did not show growth upon extended incubation. The death of *L. monocytogenes* Scott A was dependent on the combination of low pH and higher levels of PL and SDA mixture. In the present study, the pathogen died at 1% of the PL (0.56%) and SDA (0.04%) mixture at pH of 5.5 at all tested temperatures except 30 and 37°C, and 3% of PL (1.68%) and SDA (0.12%) mixture at pH of 6, indicating that the pathogen did not survive the hurdle combination of low pH and successive levels of PL and SDA mixture. Therefore, these combinations were not used for the predictive secondary growth model for *L. monocytogenes* Scott A in the present study and were identified as no-growth conditions for *L. monocytogenes* even at 37°C. At pH of 6.5 and 7.0, SGRs increased with the same levels of PL and SDA mixture (Fig. 1). However, the maximum population density was independent of the experimental conditions (Table 2).

Table 2. Conditions of temperature, pH, and potassium lactate (PL) and sodium diacetate (SDA) mixture that did not support the growth of *L. monocytogenes* Scott A.

Temperature (°C)	pH	PL/SDA concentration (%)
4	5.5	1
10	5.5	1
17	5.5	1
24	5.5	1
4	5.5	1.8
10	5.5	1.8
17	5.5	1.8
24	5.5	1.8
30	5.5	1.8
37	5.5	1.8
4	5.5	3
10	5.5	3
17	5.5	3
24	5.5	3
30	5.5	3
37	5.5	3
4	6.0	3
10	6.0	3
17	6.0	3
24	6.0	3
30	6.0	3

Legan *et al.* [12] published the growth boundary model for *L. monocytogenes* as a function of the product salt, moisture, potassium lactate, and sodium diacetate concentrations in ready-to-eat cooked meat products at 4°C. The growth boundary model shows the boundary between growth and no-growth conditions using contour plots of time to growth. More recently, predictive modeling for growth boundary of *L. monocytogenes* in lightly preserved seafoods containing diacetate and lactate at 8 and 15°C was introduced [15]. Both studies provided conditions that prevent the growth of *L. monocytogenes* in RTE products at the refrigeration temperature.

To develop the predictive models for SGR and LT, growth data in the present study were subjected to response surface analysis using the SAS General Linear Model Procedures. The resulting second and third order polynomial equations for SGR and LT are given in Table 3.

Evaluation of Model Performance

In assessing which model (quadratic or cubic) better describes the data, different approaches were employed. The first approach was to use the corresponding equations to generate predicted values for SGR and LT for each culture condition. Comparison of the best-fit values for SGR and LT as indicated by R^2 (the coefficient of determination) for both models are presented in Table 3. The values for the specific growth rate were less well predicted in the quadratic model (lower R^2 values) than the cubic model.

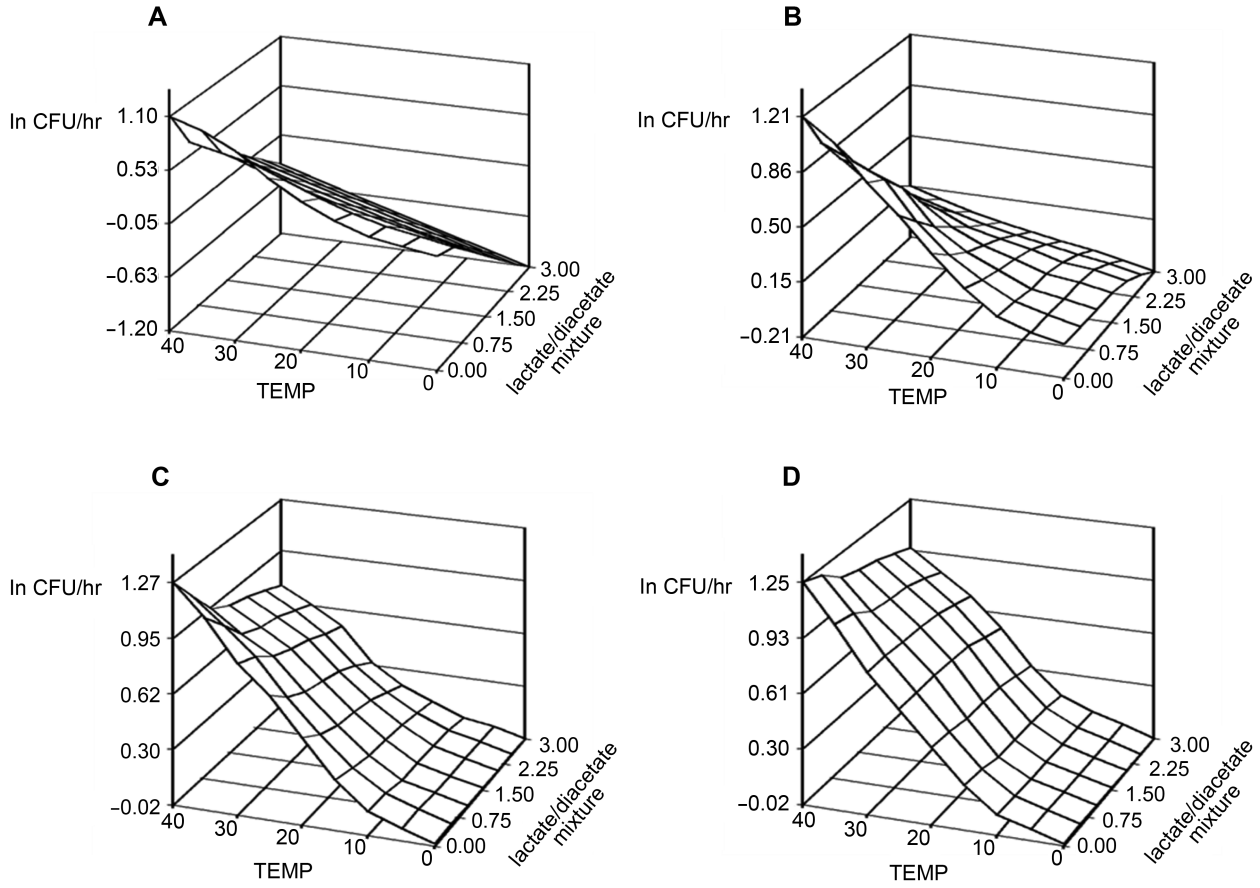

Fig. 1. Surface response models for the effects of combinations of potassium lactate and sodium diacetate on the specific growth rate of *L. monocytogenes* Scott A as a function of temperature in pH-adjusted broth.
A. pH 5.5; **B**. pH 6.0; **C**. pH 6.5; **D**. pH 7.0.

The cubic model was superior in predicting slower specific growth rates (SGR<0.05 h^{-1}). The quadratic model gave poor predictions for 4°C at pH 5.5, 6.0, and 6.5 and also poor predictions for 4 and 10°C at pH 6.5 for the concentration of PL and SDA mixture higher than 1%. However, the quadratic model for specific growth rate could

Table 3. Response surface polynomial models for specific growth rate (SGR) and lag time (LT) of *L. monocytogenes* Scott A in broth as a function of temperature, pH, and potassium lactate (PL) and sodium diacetate (SDA) concentration.

Model	R^2	Equation
Quadratic	0.914	ln y (SGR)=−0.4828−0.1814A+0.1553B−1.9967C−0.00021A^2−0.0135B^2+0.1258C^2+0.0623AB−0.0647AC+0.6089BC+0.00895ABC+0.00004511A^2B−0.000065A^2C−0.00494B^2A−0.04526B^2C+0.001147C^2A−0.02138C^2B
	0.974	ln y (LT)=36.3694+0.0843A−9.8225B+29.5501C+0.0101A^2+0.6957B^2+0.0795C^2−0.2031AB−0.0553AC−8.2605BC+0.00748ABC−0.00086A^2B+0.0000512A^2C+0.0227B^2A+0.5774B^2C
Cubic	0.983	ln y (SGR)=−2.796−0.3447A+1.5923B−2.2008C+0.00289A^2−0.2924B^2+0.0425C^2+0.1126AB−0.056AC+0.6734BC+0.00871ABC−0.000343A^2B−0.00026A^2C+0.00949B^2A−0.05146B^2C−0.0000173A^3+0.01769B^3−0.00375C^3−0.000000252A^3B^2+0.00000972A^3C^2+0.0000057B^3A^2−0.00012B^3C^2
	0.981	ln y (LT)=−220.8809−0.299A+116.6972B+20.945C+0.0163A^2−19.7216B^2+0.1314C^2−0.0531AB−0.0219AC−5.9089BC+0.0036ABC-0.00298A^2B−0.000388A^2C+0.00779B^2A+0.4274B^2C+0.0000735A^3+1.0939B^3+0.0418C^3−0.0000026A^3B^2+0.0000024A^3C^2+0.0000359B^3A^2−0.001031B^3C^2

A: Temperature.
B: pH.
C: Concentration of PL and SDA mixture.

provide acceptable predictions for SGR at temperatures above 10°C. In contrast, the R^2 values for the cubic and quadratic models developed for LT were very close (0.98 vs. 0.97), indicating no large differences in goodness-of-fit between the cubic and quadratic models for LT; that is, overall, the cubic model did not fit LT any better than the quadratic model according to the coefficient of determination.

The second approach to comparing the quadratic and cubic models was to evaluate the performance of both models for SGR and LT. Performance evaluations were carried out for data used in model development (dependent data) and for data not used in model development (independent data) but that were inside the response surface. Scatterplots of relative errors for cubic and quadratic models were developed for SGR and LT.

The quadratic model for SGR showed a regional prediction problem at low SGR (<0.05 h^{-1}) (Fig. 2A). However, the prediction error for SGR above 0.05 h^{-1} was randomly distributed around zero. The cubic model (Fig. 2B) showed a similar systematic bias in the same region (SGR<0.05 h^{-1}). Nonetheless, this prediction bias was closer to zero than the quadratic model and did not show higher RE values. This observation was further confirmed using the %RE parameter of Oscar [17]. The boundaries of that method are equivalent to those proposed by Ross *et al.* [20] for accepted values of B_f for generation times, which are 0.7 (fail-safe) to 1.15 (fail-dangerous), as shown in Figs. 2A and 2B. The zone was wider in the fail-safe direction because greater prediction error can be tolerated in the fail-safe direction when models are used to predict food safety [20]. Overall, the poorer performance of the quadratic model for SGR, mainly at lower SGR, was attributed to a regional prediction problem that was corrected by increasing the order of the model to the cubic level. Compared with the quadratic model, the cubic model for SGR increased the %RE from 74.7% to 92.9% and from 85.7% to 92.6% for dependent and independent data, respectively (Table 4). In contrast, the cubic model for LT decreased the %RE inside the acceptable prediction zone from 93.9% to 92.9% and from 96.4% to 92.8% for dependent and independent data, respectively (Table 4). These results indicated that the quadratic model provided better predictions of LT than the cubic model.

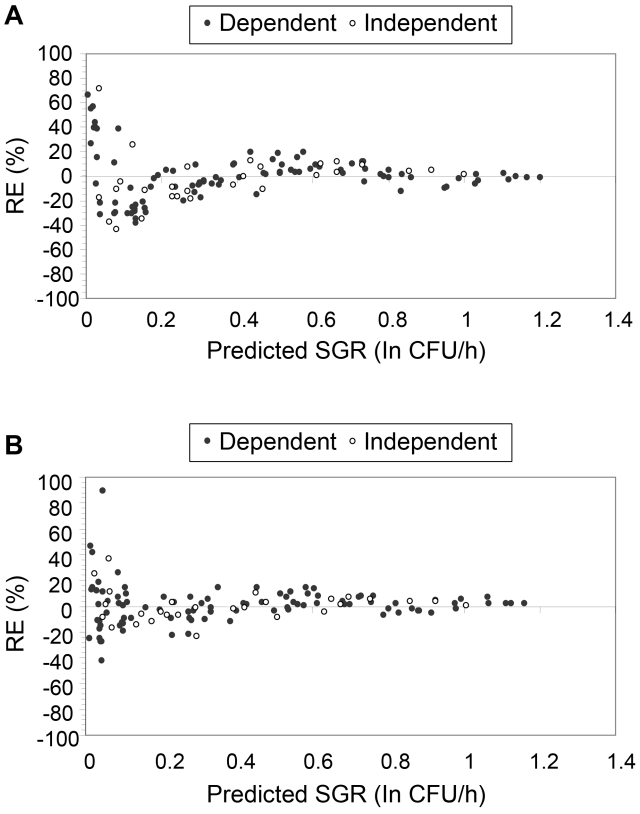

Fig. 2. Relative error (RE) plot with an acceptable prediction zone for specific growth rate (SGR) of *L. monocytogenes* Scott A in broth using a quadratic model (**A**) and cubic model (**B**) for dependent data used in model development and independent data used for model validation.

The last approach to evaluate the performance of the models was to use the prediction bias (B_f) and accuracy factors (A_f) [19]. The quadratic and cubic models for SGR had a B_f of 1.00 and 1.00 for dependent, and 1.08 and 1.00 for independent data, respectively. For LT, the B_f values of quadratic and cubic models were 1.00 and 1.00 for dependent, and 0.96 and 0.99 for independent data, respectively (Table 4). Ross *et al.* [20] recommended that for models describing pathogen growth rate, B_f in the range of 0.9 to 1.05 could be considered good, 0.7 to 0.9 or 1.06 to 1.15 to be considered acceptable, and less than 0.7 or greater than 1.15 be

Table 4. Performance of growth models for *L. monocytogenes* Scott A in brain heart infusion broth based on prediction bias (B_f) and accuracy factors (A_f), and the percentage of relative errors (%RE) in the acceptable prediction zone.

Data set	Growth parameter	n	Growth medium	Quadratic			Cubic		
				B_f	A_f	%RE	B_f	A_f	%RE
Dependent	SGR	99	Broth	1	1.19	74.7	1	1.1	92.9
Dependent	LT	99	Broth	1	1.17	93.9	1	1.17	92.9
Independent	SGR	28	Broth	1.08	1.16	85.7	1	1.08	92.6
Independent	LT	28	Broth	0.96	1.07	96.4	0.99	1.09	92.8

considered unacceptable. For A_f, the cubic model for SGR had a lower A_f of 1.10 and 1.08 for dependent and independent data, respectively, than the quadratic model, which had A_f of 1.19 and 1.16 for dependent and independent data, respectively. This indicated that the cubic model shows a better performance than the quadratic model for SGR data in the present study. In contrast, the A_f values for LT were almost similar for quadratic and cubic model in the present study (Table 4). Ideally, predictive models would have A_f and B_f of 1.00, but typically, the accuracy factor will increase by 0.10 to 0.15 for every variable in the model [19]. Thus, an acceptable model that predicts the effect of temperature, pH, and PL and SDA mixture on SGR and LT for *L. monocytogenes* could be expected to have an A_f of 1.3 to 1.45. The %RE method in the present study also evaluated the performance of model predictions well, particularly when regional prediction problems occurred as observed in the quadratic model for SGR (Fig. 2A). When a model shows underprediction in one region of the response surface and overprediction in another region of the response surface, acceptable B_f and A_f were observed and no differences were observed between the quadratic and the cubic models. When the RE plot was examined (Fig. 2A), it was found that the broth model provided overly fail-dangerous predictions at short SGR and slightly fail-safe but not overly fail-safe predictions at longer SGR.

In Table 5, we compare the observed growth kinetics of *L. monocytogenes* Scott A in static broth without Purasal P Opti.Form 4 from the present study with those predicted by the USDA pathogen modeling program [25] under aerobic and anaerobic conditions as a function of temperature (4, 10, 17, 24, 30, 37°C) and pH (5.5, 6.0, 6.5, 7.0). At 4 and 10°C, the predicted SGRs of *L. monocytogenes* Scott A cells in broth under anaerobic conditions by PMP were longer than those under aerobic conditions, regardless of the pH. On the other hand, faster SGRs under aerobic conditions than those under anaerobic conditions were predicted by PMP at the temperature above 17°C. In addition, a large discrepancy between the observed data in the present study and the predicted data by PMP was noticed, especially in SGR, regardless of the growth conditions. At 37°C and pH 7.0, the predicted SGR by PMP under aerobic condition

Table 5. Comparison of observed growth kinetics of *L. monocytogenes Scott A* in static broth with those of predicted by pathogen modeling program (PMP) under aerobic and anaerobic conditions as a function of temperature and pH.

Temperature (°C)	pH	SGR (log CFU/hr)			LT(hr)		
4	5.5	0.013[a]	0.03[b]	0.058[c]	157.92[a]	128.9[b]	96.9[c]
	6.0	0.015	0.047	0.071	68.4	79.2	65.0
	6.5	0.016	0.06	0.075	53.1	62.0	50.8
	7.0	0.014	0.062	0.071	45.9	62.0	46.5
10	5.5	0.042	0.095	0.136	37.5	47.3	33.6
	6.0	0.048	0.144	0.173	21.7	29.4	22.3
	6.5	0.051	0.178	0.193	15.5	23.3	17.3
	7.0	0.040	0.182	0.193	7.2	23.5	15.7
17	5.5	0.120	0.277	0.289	7.3	17.5	13.0
	6.0	0.148	0.408	0.385	7.3	11.0	8.5
	6.5	0.150	0.495	0.462	4.3	8.8	6.5
	7.0	0.131	0.495	0.462	4.7	9.0	5.9
24	5.5	0.222	0.63	0.495	3.8	7.8	6.8
	6.0	0.267	0.866	0.693	1.8	5.0	4.4
	6.5	0.285	0.99	0.866	4.1	4.0	3.4
	7.0	0.244	0.99	0.866	2.1	4.2	3.0
30	5.5	0.350	0.99	0.578	2.3	4.5	5.0
	6.0	0.368	1.386	0.866	1.5	2.9	3.2
	6.5	0.365	1.733	1.155	1.4	2.4	2.4
	7.0	0.345	1.733	1.155	1.4	2.5	2.1
37	5.5	0.453	1.386	0.578	2.0	2.9	4.6
	6.0	0.491	1.733	0.866	1.7	1.9	3.0
	6.5	0.458	2.31	1.155	1.3	1.6	2.2
	7.0	0.497	2.31	1.386	1.2	1.7	1.9

[a]Indicates the observed data without Purasal P Opti.Form 4 from the present study.
[b]Indicates the predicted data under aerobic condition from PMP.
[c]Indicates the predicted data under anaerobic condition from PMP.

was five times faster than the observed SGR in the present study. In general, shorter LTs were observed in the present study, compared with the predicted LTs by PMP, except at 4°C and pH 5.5. It was reported that the agitation culture during the development of growth model in PMP results in the overestimation of microbial growth rates in foods [28].

Overall, cubic polynomial models for SGR and LT provided acceptable predictions of *L. monocytogenes* growth in the matrix of conditions described in the present study, with both dependent and independent data, and can be used as a tool to estimate the impact of food formulation containing a potassium lactate and sodium diacetate mixture (0 to 3%) and storage conditions of pH (5.5–7.0) and temperature (4–37°C) on the growth of *L. monocytogenes* in the retail market. The models will be incorporated into the Pathogen Modeling Program for use in the food industry. However, the models developed in this study require further validation in different food products to test the ability of the models to predict the growth of *L. monocytogenes* in different food matrices. In addition, the secondary models require further evaluation for model performance at pH and temperatures outside (extrapolation) the current model boundaries.

Acknowledgments

Funding for this study was provided by the JIFSAN (Joint Institute of Food Safety and Nutrition). Thanks are extended to PURAC America Inc. for providing PURASAL P Opti. Form 4 for this study.

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