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Predictive model for growth of *Bacillus cereus* during cooling of cooked rice

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ABSTRACT

Bacillus cereus is frequently implicated in foodborne outbreaks associated with the consumption of cooked rice. The main contributing factors leading to outbreaks is rice cooked in large quantities and subsequently, inadequately chilled or stored at room temperatures for a prolonged period of time prior to consumption. *Bacillus cereus* growth in cooked rice inoculated with approximately 2 log CFU/g of heat-shocked (80 °C/10 min) spores at several isothermal conditions (between 10 and 49 °C) was quantified. *B. cereus* populations were determined by plating on mannitol egg yolk polymyxin agar and incubating at 30 °C for 24 h. Primary growth models, namely Baranyi, Huang, modified Gompertz, and logistic models were fitted to growth data. Specific growth rates from all four primary models were used to fit the modified Ratkowsky square-root model with respect to temperature. All four primary models were well fitted by the modified Ratkowsky model (R^2 values from 0.90–0.99). Based on the goodness of fit secondary model statistics (R^2, SSE, RMSE), the Baranyi model performed the best and was chosen for tertiary modeling. Acceptable prediction zone (APZ) analysis was performed for validation of the Baranyi model predictions during single rate exponential and biphasic linear cooling temperature profiles. For single rate cooling, 23 of the 24 predictions fell within the APZ (−1.0 to 0.5 log CFU/g). For biphasic linear cooling, 26 of the 28 predictions fell within the APZ. The developed dynamic model can be used to predict potential *B. cereus* growth from spores in cooked rice during chilling and thus, support the disposition of product subject to cooling deviations.

1. Introduction

Bacillus cereus, a spore-forming pathogen of public health significance for the last 40 years, is a common inhabitant of soil and may find its way into crops and hence into foods. The pathogen has been isolated from rice dishes, raw and pureed vegetables, dairy products, spices, food crops, and cooked, ready-to-eat foods (Eglezos et al., 2010; Fangio et al., 2010; Kramer and Gilbert, 1989; Pao et al., 2006; Rajkovic et al., 2005). While raw husked rice has been found to contain 2.5×10^1 CFU/g, raw unhusked rice can contain as high as 2.5×10^3 CFU/g of *Bacillus cereus* spores (Sarrias et al., 2003). In a survey conducted in retail foods, about 52.8% of the total 178 samples of raw rice were reported to be contaminated with spores of *B. cereus* (Ankolekar et al., 2009). In another survey, *B. cereus* was isolated from 54 of 136 samples of rice and their processed products (Jang et al., 2006). Thus, sufficient evidence exists to document that rice can be contaminated with spores of *B. cereus*.

B. cereus is an etiological agent of diarrheal as well as emetic foodborne illness syndromes. The pathogen is one of the main microorganisms, implicated in an estimated 63,400 episodes of foodborne illnesses annually in the United States (Scallan et al., 2011). Consumption of cooked rice is one of the main foods, frequently implicated in foodborne outbreaks with *B. cereus* and widely reported (Delbrassinne et al., 2015; Pao et al., 2006). The main contributing factors leading to outbreaks is rice cooked in large quantities in advance of a social event or a large gathering and inadequately chilled after cooking or stored at room temperatures for an extended period of time prior to consumption (Adams and Moss, 2000; Mossel et al., 1991).

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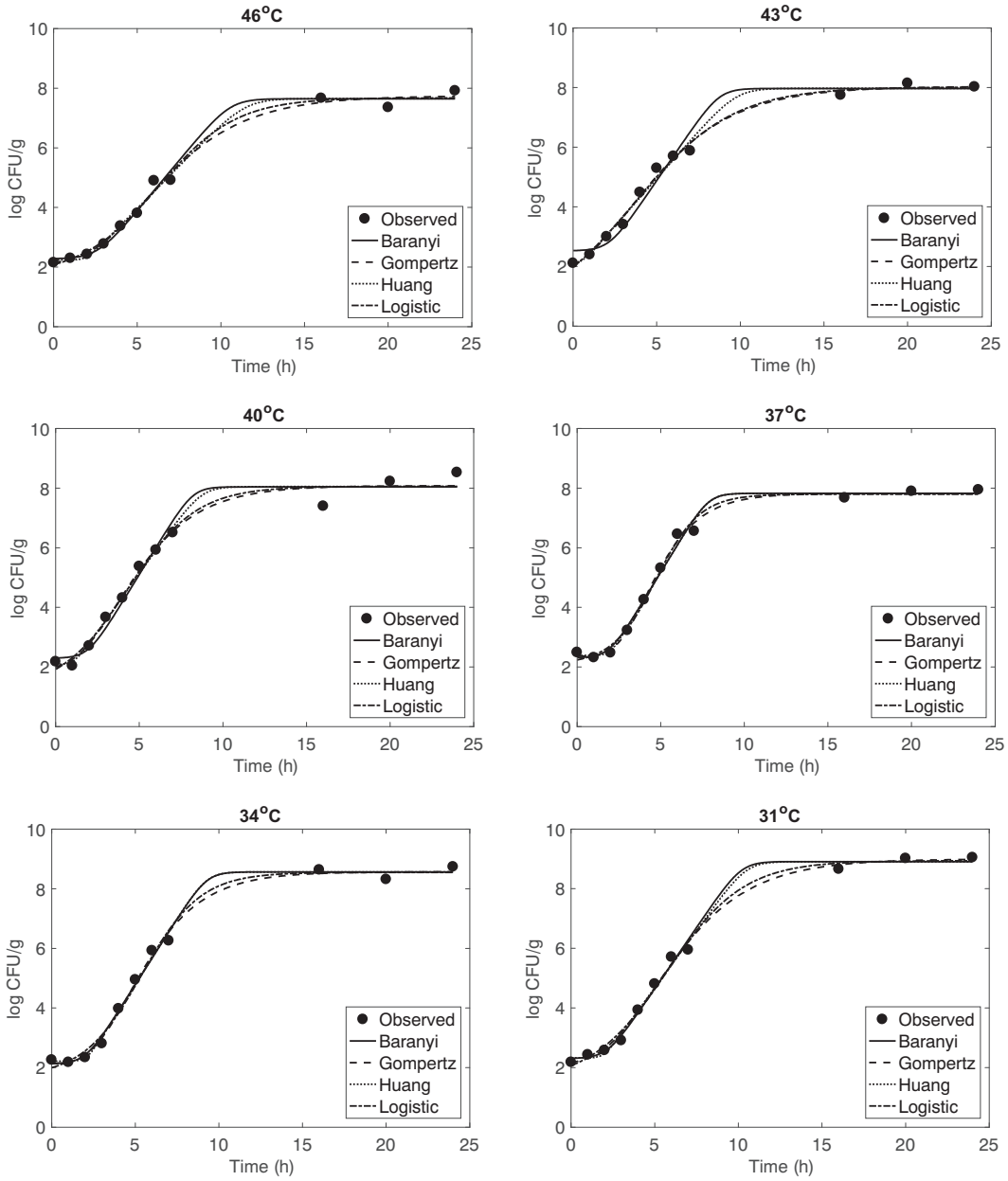

Fig. 1. Observed growth of *B. cereus* in cooked rice stored at different temperatures and fitted primary growth models. Each graph illustrates a representative trial for each temperature point.

Bryan et al. (1981) reported that the highest temperature achieved during cooking rice is 99 °C. While *B. cereus* vegetative cells are destroyed at this cooking temperature, spores are likely to survive since the decimal reduction time (D-value) at 100 °C ranged from 2.2–5.5 min to 80 min at 80 °C, depending on the strains of *B. cereus* (Montville et al., 2005). The heat activated spores can germinate, outgrow and multiply to potentially dangerous population levels (about 5–6 log CFU/g) or produce a heat-stable toxin (emetic toxin) in food (Little et al., 2002). After cooking rice, a public health hazard may occur because of inadequate rate and extent of cooling or storage at improper temperatures, such as storage at temperatures > 5 °C (41 °F) for > 4 h. Reheating precooked rice prior to consumption would not destroy the preformed toxin responsible for the emetic type of illness and may not sufficiently reduce the vegetative cell numbers to prevent diarrheal syndrome. Approximately 95% of the emetic syndrome outbreaks are primarily caused by the consumption of cooked or fried rice and is frequently reported in countries, such as Japan, where rice is a staple food (Granum, 2005, 2007; Kramer and Gilbert, 1989).

Predictive microbial modeling programs such as the USDA Pathogen Modeling Program (PMP) and ComBase Predictor are extensively used by the regulatory agencies as well as the food industry to predict the behavior of pathogens in foods under conditions relevant to the food processing operations. While these user-friendly modeling tools include the cooling models to predict the relative growth of *Clostridium perfringens* or *Clostridium botulinum* from spores at temperatures applicable to the cooling of cooked foods, dynamic models for *B. cereus* are not currently available. Currently, there are only two growth models available for *B. cereus*, both of which have limitations in evaluating cooling deviations involving nonmeat components (e.g., rice, pasta, beans, etc.). These models are the ARS PMP 7.0/8.0 growth model for *B. cereus* and the ComBase Predictor growth model for *B. cereus*. The PMP 7.0/8.0 growth model for *B. cereus* has the following limitations to the model: Growth experiments conducted in broth culture not product; predictions based on static temperature conditions not dynamic

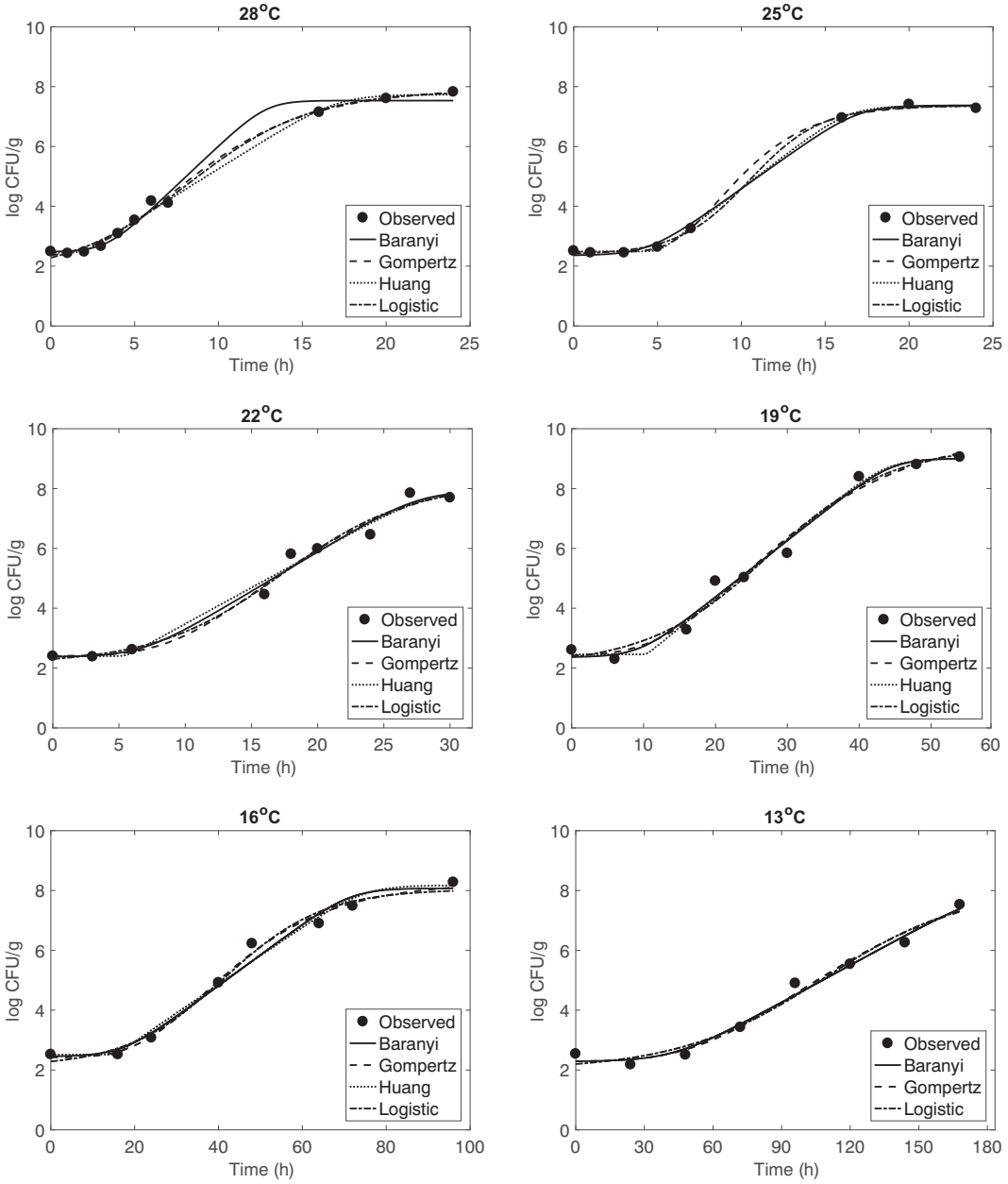

Fig. 1. (*continued*)

Table 1
Modified Ratkowsky model parameters and goodness of fit statistics for each primary growth model used in the current study.

Primary model	*a*	*b*	T_{min}	T_{max}	RMSE	R^2
Baranyi	0.0046 (0.0009, 0.0083)	0.0656 (−0.0007, 0.1319)	8.67 (5.09, 12.25)	49.68 (48.14, 51.22)	0.080	0.991
Gompertz	0.0053 (−0.0125, 0.0230)	0.0444 (−0.1270, 0.2158)	5.90 (−6.54, 18.34)	49.46 (44.99, 53.94)	0.264	0.903
Huang	0.0028 (−0.0018, 0.0074)	0.1069 (−0.1030, 0.3168)	5.77 (−6.58, 18.12)	48.47 (45.64, 51.30)	0.200	0.925
Logistic	0.0055 (−0.0030, 0.014)	0.0815 (−0.0601, 0.2231)	5.42 (−4.25, 15.09)	47.86 (45.91, 49.80)	0.312	0.938

T represents the temperature in °C; T_{min} and T_{max} are theoretical growth limits in °C; *a* and *b* are regression coefficients; RMSE is the root mean square error; R^2 is the coefficient of determination. The 95% confidence bounds are presented for each estimated parameter value.

temperature conditions; model only provides prediction for temperatures up to 107.6 °F (42 °C), but *B. cereus* can grow up to a temperature of 131 °F (55 °C); model not fail-safe for predicting lag phase; and the growth model has never been validated for food products. Whereas, the ComBase Predictor growth model for *B. cereus* has the following limitations to the model: Growth experiments conducted in broth culture not product; model only provides prediction for temperatures up to 93.2 °F (34 °C), but *B. cereus* optimal growth occurs from 86 °F (30 °C) to 104 °F (40 °C), with a maximum growth temperature of 131 °F (55 °C); model not fail-safe for predicting lag phase; and the growth model has never been validated for food products. Accordingly, a dynamic model for growth of *B. cereus* from spores at temperatures applicable to the cooling of cooked beans was recently developed (Juneja et al., 2018). This study was carried out to develop a dynamic predictive model for *B.*

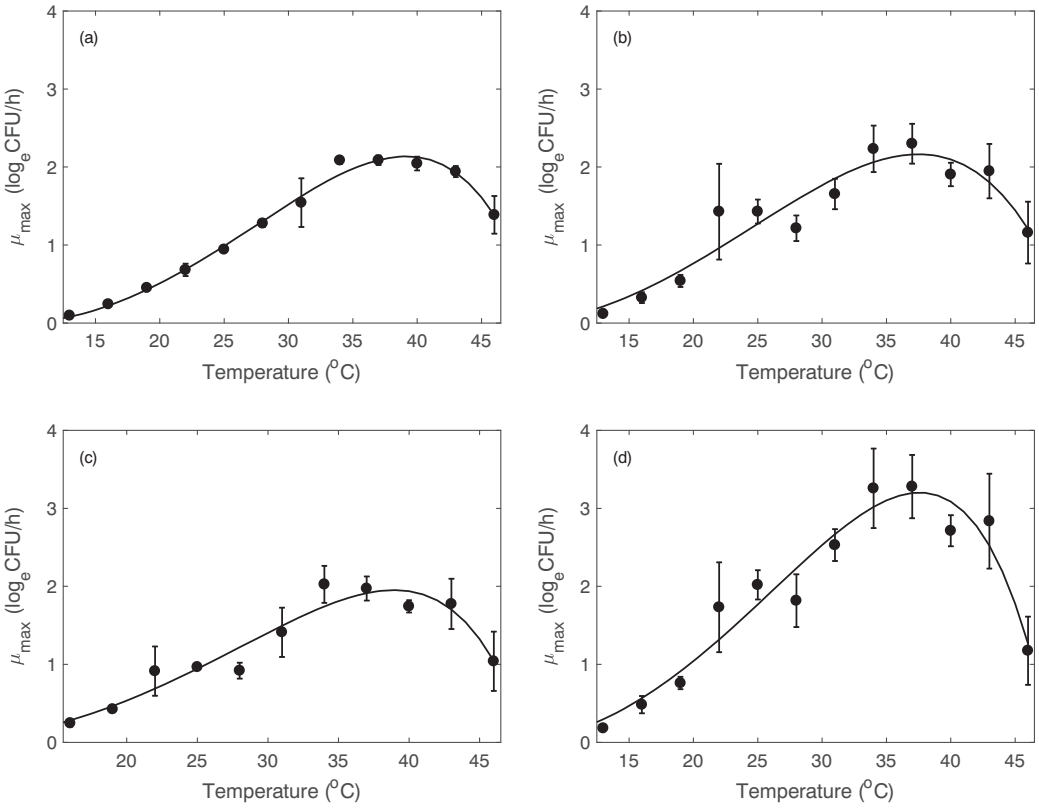

Fig. 2. Modified Ratkowsky predicted μ_{max} values generated from the (a) Baranyi, (b) modified Gompertz, (c) Huang, and (d) logistic primary growth model fitted isothermal curves compared with the mean observed (n = 3) values. The solid line represents the predicted values and the dots represent the mean observed values.

Table 2
Hyperbolic function parameters and goodness of fit statistics for each primary growth model used in the current study.

Primary model	*p*	*q*	RMSE	R^2
Baranyi	32.22 (29.36, 35.08)	4.429 (3.650, 5.209)	0.671	0.997
Gompertz	41.34 (33.79, 48.90)	2.698 (0.762, 4.635)	1.910	0.987
Huang	26.58 (17.68, 35.47)	6.936 (3.762, 10.110)	1.213	0.954
Logistic	49.32 (44.70, 53.94)	1.918 (0.855, 2.982)	1.503	0.997

T represents the temperature in °C; *p* is a parameter that accounts for the decrease in lag time as temperature increases; *q* represents the temperature (°C) at which lag time is infinite; RMSE is the root mean square error; R^2 is the coefficient of determination. The 95% confidence bounds are presented for each estimated parameter value.

cereus in cooked rice and to validate the model using exponential and biphasic linear temperature profiles. This cooling model will be incorporated in the USDA-PMP and will enable the industry and regulatory agencies to evaluate the safety of cooked rice after cooling and thus, with the disposition of products subject to cooling deviations. Furthermore, this model will aid the industry in designing risk-based preventative controls and HACCP systems, including by aiding in designing critical control limits as well as lower operating costs, and guard against the hazards associated with excessive growth of *B. cereus* in cooked rice.

2. Materials and methods

2.1. Test organisms

A four-strain cocktail, consisting of *Bacillus cereus* strains NCTC 11143[4810/72] and Mac 1 (emetic strains isolated from cooked rice and mac and cheese, respectively), 935A/74 and Brad 1 (diarrheal strains isolated from turkey loaf and canned soup, respectively) were used in the present study. These strains, obtained from the Eastern Regional Research Center (Wyndmoor, PA) culture collection, were preserved at −20 °C in sterile distilled water (dH_2O).

2.2. Spore production

B. cereus stock cultures were activated by transferring a loopful of each strain in 10 mL brain heart infusion (BHI) broth tubes and incubating overnight at 37 °C. Spore suspensions of the cultures were produced in NAMS agar [nutrient agar (NA; Difco, Becton, Dickinson and Co., Sparks, MD) + 0.05 g/L of manganese sulfate ($MnSO_4$; Sigma-Aldrich Co., St. Louis, MO)], as described previously (Juneja et al., 2018). A spore cocktail containing all four strains of *B. cereus* was prepared by mixing an equal number of spores of *B. cereus* from each of the four suspensions and heat shocked for 10 min at 80 °C immediately prior to experimentation.

2.3. Product

Extra-long grain rice was obtained from a local retail grocery store. After washing rice, one cup of rice along with water (1:2) were placed in a rice cooker (Aroma Simply Stainless Rice Cooker, Model No. ARC-753SG). After covering with a lid, rice was cooked for about 20 min. The cooked rice was left at room temperature to cool in a period of about an hour.

2.4. Sample preparation

Cooked rice samples (5 g) were weighed into sterile filter bags (Whirl-Pak bags, 7-oz [207-mL] capacity; 3.0 mil [0.076 mm] thick; product no. B01385; Nasco, Ft. Atkinson, WI) and inoculated with 100 µL of an appropriate dilution of the heat-shocked (80 °C/10 min) *B.*

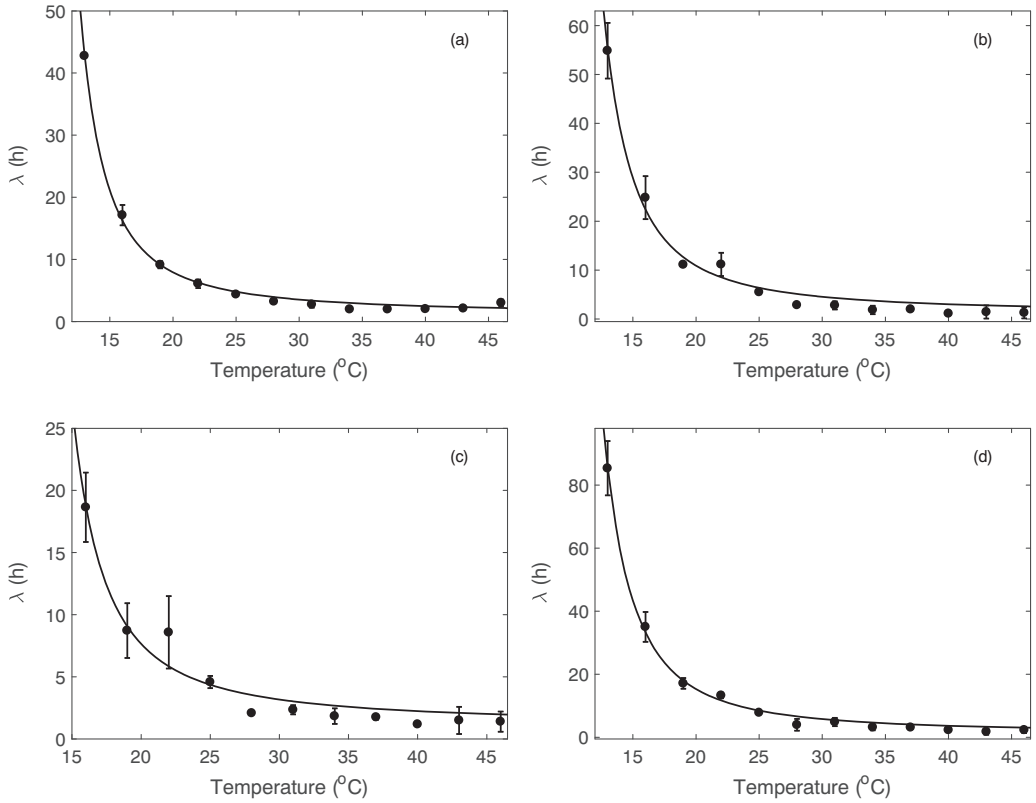

Fig. 3. Hyperbolic function predicted λ values generated from the (a) Baranyi, (b) modified Gompertz, (c) Huang, and (d) logistic primary growth model fitted isothermal curves compared with the mean observed (n = 3) values. The solid line represents the predicted values and the dots represent the mean observed values.

Table 3
Concentration of *B. cereus* in cooked rice before and after each dynamic cooling profile.

Profile		Initial concentration (log CFU/g, mean ± standard deviation)	Final concentration observed (log CFU/g, mean ± standard deviation)
Exponential cooling	6 h	2.33 ± 0.07	2.65 ± 0.04
	9 h	2.56 ± 0.09	3.62 ± 0.03
	12 h	2.45 ± 0.08	4.46 ± 0.14
	15 h	2.17 ± 0.18	5.15 ± 0.11
	18 h	2.53 ± 0.02	5.80 ± 0.11
	21 h	2.53 ± 0.08	6.48 ± 0.03
Biphasic linear cooling	54.5–27 °C (6.5 h)	2.39 ± 0.06	4.28 ± 0.24
	54.5–27 °C (5 h) + 27–7.2 °C (1.5 h)	2.28 ± 0.09	3.74 ± 0.30
	54.5–27 °C (4 h) + 27–7.2 °C (2.5 h)	2.27 ± 0.18	3.30 ± 0.27
	54.5–27 °C (3 h) + 27–7.2 °C (3.5 h)	2.42 ± 0.06	3.23 ± 0.36
	54.5–27 °C (2.5 h) + 27–7.2 °C (4 h)	2.45 ± 0.10	2.91 ± 0.08
	54.5–27 °C (2 h) + 27–7.2 °C (4.5 h)	2.57 ± 0.08	3.05 ± 0.17
	54.5–27 °C (1.5 h) + 27–7.2 °C (5 h)	2.54 ± 0.04	2.86 ± 0.10

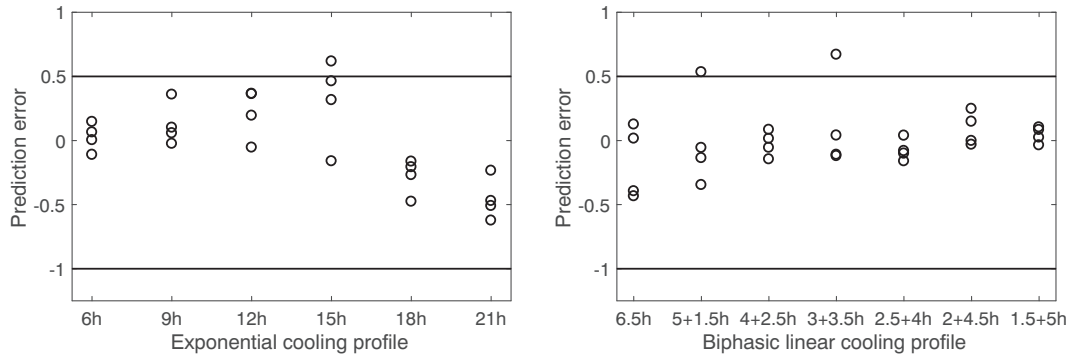

Fig. 4. Acceptable prediction zone analysis (APZ) for (a) single-rate exponential cooling, and (b) biphasic linear cooling. X-axis labels refer to the different cooling profiles used.

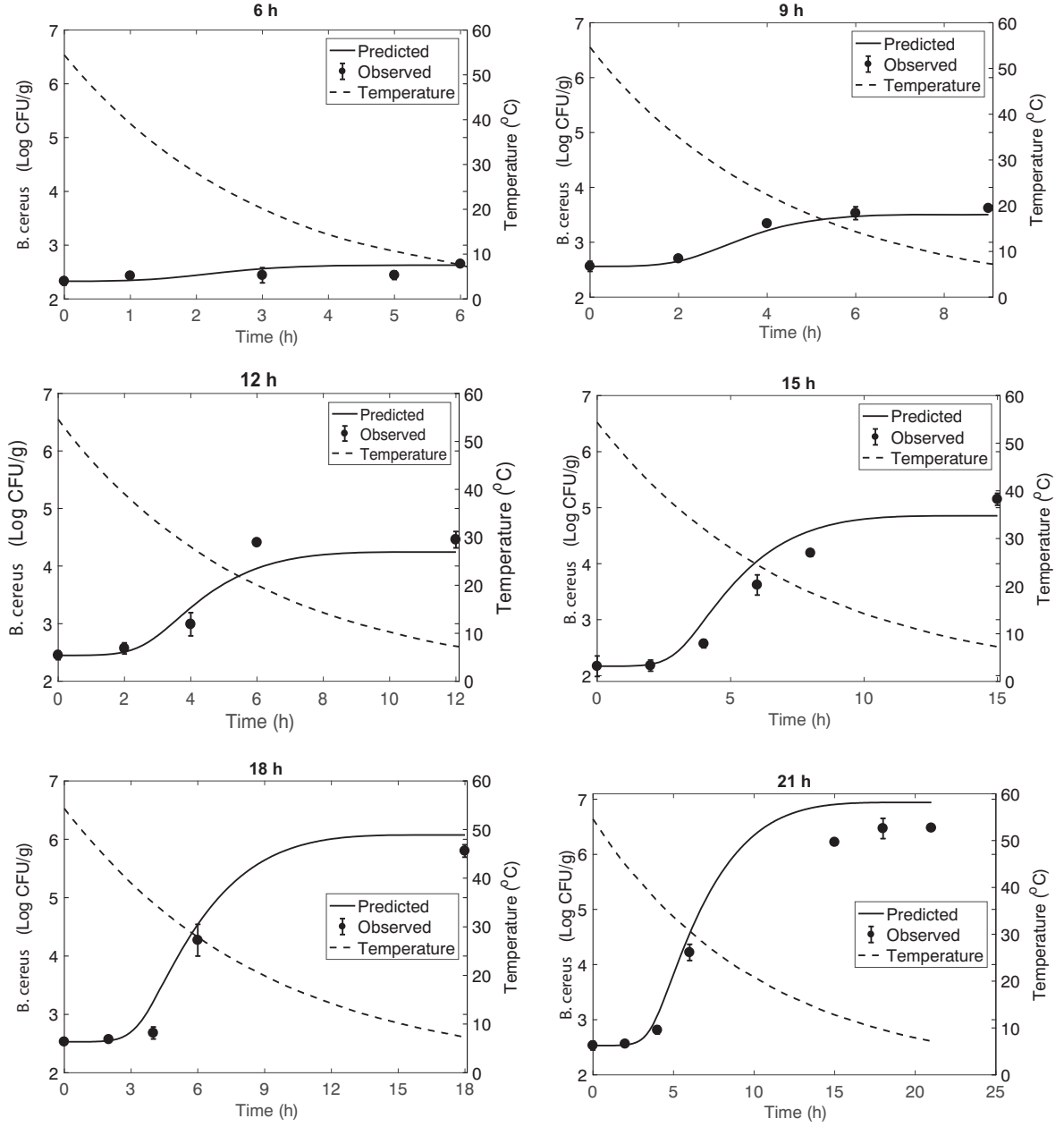

Fig. 5. Baranyi-predicted and mean (n = 4) observed *B. cereus* growth behavior during the single-rate exponential cooling experiment. The observed temperature during each experiment is illustrated with a dashed line.

cereus spore mixture so that the final concentration was approximately 2.0 log spores/g. Thereafter, the inoculated rice in bags were manually mixed for about 2 min to ensure uniform distribution of spores in the rice. The rice bags were gently compressed to remove air and were closed by twice folding over the aluminum wire ends. Non-inoculated bags containing rice were used as controls.

2.5. Growth of B. cereus at isothermal conditions

The static temperature covering the entire biokinetic growth temperature ranged from 10 to 49 °C and the growth was quantified in an incubator (Lab-Line Imperial III Incubator, Labline Instruments, Inc., Melrose Park, IL). The temperature for all isothermal temperatures were monitored using a wireless thermocouple data logger (Madge Tech 4, Madge Tech, Inc., Warner, NH). Three trials were performed for each temperature. For each trial, the sampling frequency was pre-determined and based on the predictions from the ARS-Pathogen Modeling Program and ranged from every 7 days at 10 °C to 3 to 24 h at 49 °C. Total sampling time ranged from 28 days at 10 °C to 96 h at 49 °C.

2.6. Dynamic temperature profiles

To assess performance of the dynamic model, data was collected under dynamic conditions. Rice sample bags at room temperature (22–23 °C) were placed in an incubator or circulating water bath (NESLAB RTE-221, NESLAB Instruments, Inc., Newington, NH) programmed to continuously decrease temperatures from 54.5 to 7.2 °C. After the rice samples reached 54.5 °C in 10 min, the cooling experiments, for exponential rate of cooling, were performed in a water bath programmed to decrease temperature from 54.5 to 27 °C in 6, 9, 12, 15, 18, or 21 h. Two replications, each performed in duplicate, were conducted with at least four samples at specific intervals during the exponential chilling collected. For biphasic linear prescribed cooling from 54.5 to 7.2 °C in 6.5 h, cooling time was varied as under: 54.5–27 °C in

6.5 h; 54.5–27 °C in 5 h + 27–7.2 °C in 1.5 h; 54.5–27 °C in 4 h + 27–7.2 °C in 2.5 h; 54.5–27 °C in 3 h + 27–7.2 °C in 3.5 h; 54.5–27 °C in 2.5 h + 27–7.2 °C in 4 h; 54.5–27 °C in 2 h + 27–7.2 °C in 4.5 h; and 54.5–27 °C in 1.5 h + 27–7.2 °C in 5 h. Two trials of these dynamic cooling experiments were performed and duplicate bags were sampled immediately before and after cooling. Both exponential rate cooling and biphasic linear cooling data were used to evaluate the performance of the predictive model developed in the present study. The temperature for both exponential and dynamic profiles were recorded using a wireless thermocouple data logger, as used for isothermal temperatures.

2.7. Enumeration of bacteria

Sterile peptone water (0.1% w/v) was added to the samples to obtain a 1:1 (wt/vol) slurry and then, homogenized in a stomacher (MiniMix 100, Interscience, Rockland, MA) for 2 min. Serial dilutions of the liquid portions were done, followed by surface plating appropriate dilutions, in duplicate, onto MYP agar plates which were subsequently incubated for 24 h at 30 °C. Total population of *B. cereus* at each sampling time were recorded as log CFU per gram of rice. Uninoculated samples (negative controls) were used to ensure that the naturally occurring *B. cereus* were not present.

2.8. Primary models

Four commonly used primary growth models were fitted to the *B. cereus* growth data: Baranyi, modified Gompertz, Huang, and logistic models (Baranyi and Roberts, 1994; Gibson et al., 1987; Huang, 2008). Primary models were fitted using MATLAB (Version R2017a, Mathworks, Natick, MA) nonlinear least squares curve fitting toolbox with trust-region algorithm. Growth data were transformed to $\log_e$ CFU/g units prior to model fitting.

The Baranyi model (Baranyi and Roberts, 1994) was used to fit the sigmoidal shape of the bacterial growth curve:

$$y_t = y_o + \mu_{max} F(t) - ln\left(1 + \frac{e^{\mu_{max} F(t)} - 1}{e^{y_{max} - y_o}}\right) \quad (1)$$

where

$$F(t) = t + \frac{1}{v}\ln(e^{-vt} + e^{-h_o} - e^{(-vt-h_o)}) \quad (2)$$

y_t represents the cell concentration in $\log_e$ CFU/g at time t; y_o represents the initial cell concentration in $\log_e$ CFU/g; y_{max} represents the maximum cell concentration in $\log_e$ CFU/g; μ_{max} is the maximum specific growth rate in $\log_e$ CFU/h; v is the rate of increase in the limiting substrate, assumed to be equal to μ_{max}; λ is the duration of the lag phase in hours; h_o is equal to $\mu_{max}\lambda$.

Initially, four parameters of the Baranyi model were estimated with respect to the growth data: y_o, y_{max}, μ_{max}, h_o. The average value of h_o across all temperatures was then calculated. The other three parameters were then estimated again with the fixed h_o value (Juneja et al., 2007).

The modified Gompertz and logistic models are empirical primary models commonly used to model the growth of bacteria. They are represented by the following equations, respectively (Gibson et al., 1987):

$$y_t = A + C\ \exp(-\exp(-B(t - M)) \quad (3)$$

$$y_t = A + \frac{C}{1 + e^{-B(t-M)}} \quad (4)$$

where y_t represents the cell concentration in $\log_e$ CFU/g at time t; A represents the asymptotic log count as t decreases indefinitely, equal to $\log_e(x_{max}/x_o)$; B represents the growth rate at M, where M is the time at which the growth rate is at a maximum in hours; C is the asymptotic log count as t increases indefinitely. Growth parameters were calculated from the modified Gompertz model parameters as follows:

$$\lambda = M - \frac{1}{B} \quad (5)$$

$$\mu_{max} = \frac{BC}{e} \quad (6)$$

Similarly, growth parameters were calculated from the logistic model parameters as follows:

$$\lambda = M - \frac{2}{B} \quad (7)$$

$$\mu_{max} = \frac{BC}{4} \quad (8)$$

The Huang model (Huang, 2008) was also used to model the growth of *B. cereus* in cooked rice:

$$y_t = y_o + y_{max} - ln\{e^{y_o} + [e^{y_{max}} - e^{y_o}]e^{\mu_{max} B(t)}\}, \quad (9)$$

where

$$B(t) = t + \frac{1}{\alpha} ln \frac{1 + e^{-\alpha(t-\lambda)}}{1 + e^{\alpha\lambda}} \quad (10)$$

y_t represents the cell concentration in $\log_e$ CFU/g at time t; y_o represents the initial cell concentration in $\log_e$ CFU/g; y_{max} represents the maximum cell concentration in $\log_e$ CFU/g; μ_{max} is the maximum specific growth rate in $\log_e$ CFU/h; λ is the duration of the lag phase in hours. An alpha value of 4 was used as recommended by Huang (2013). The Huang model was not able to be fitted to growth data at 13 °C.

2.9. Secondary models

The modified Ratkowsky secondary model (Ratkowsky et al., 1983) was fitted to the maximum specific growth rates (μ_{max}) for each primary model to analyze the temperature effect on μ_{max} (Zwietering et al., 1991):

$$\mu_{max} = a(T - T_{min})^2\{1 - exp[b(T - T_{max})]\} \quad (11)$$

where T represents the temperature in °C; T_{min} and T_{max} are theoretical growth limits in °C; a and b are regression coefficients.

To describe the effect on the lag phase, a hyperbolic function as described by Zwietering et al. (1991) was used:

$$\lambda = e^{\frac{p}{T-q}} \quad (12)$$

where T represents the temperature in °C; p is a parameter that accounts for the decrease in lag time as temperature increases; q represents the temperature (°C) at which lag time is infinite.

Secondary models were fitted using MATLAB (Version R2017a, Mathworks, Natick, MA) nonlinear least squares curve fitting toolbox with trust-region algorithm.

2.10. Goodness of fit statistics

Goodness of fit statistics were used to compare model performance of both primary and secondary models, as well as in validation of the generated dynamic model. Statistics used in analysis included accuracy factor and bias factor (Ross, 1996), root mean square error (RMSE) (Chai and Draxler, 2014), sum of squared errors of prediction (SSE), and coefficient of determination (R^2) as indicated by the following equations:

$$\text{Accuracy factor} = 10^{\frac{\sum\left|\log\left(\frac{GT_{predicted}}{GT_{observed}}\right)\right|}{n}} \quad (13)$$

$$\text{Bias factor} = 10^{\frac{\sum\log\left(\frac{GT_{predicted}}{GT_{observed}}\right)}{n}} \quad (14)$$

$$\text{RMSE} = \sqrt{\frac{1}{n}\sum_{i=1}^{n} e_i^2} \quad (15)$$

$$SSE = \sum_{i=1}^{n} e_i^2 \quad (16)$$

where $GT_{predicted}$ represents the predicted generation time (h), $GT_{observed}$ represents the observed generation time (h), n represents sample size, and e represents model prediction error.

2.11. Tertiary models

The following first-order differential equations proposed by Baranyi and Roberts (1994) were used to predict microbial growth based on dynamic temperature profiles:

$$\frac{dy}{dt} = \frac{1}{1 + e^{-Q(t)}}\mu_{max}(T(t))(1 - e^{(y(t)-y_{max})}) \quad (17)$$

$$\frac{dQ}{dt} = \mu_{max}(T(t)) \quad (18)$$

where $y(0) = y_o$ and $Q(0) = \ln(Q_o)$ are the initial conditions. These equations were solved by utilizing the fourth-order Runge-Kutta method by using MATLAB software. Once solved, microbial growth predictions could be made based off dynamic time-temperature profiles.

2.12. Validation

Acceptable prediction zone (APZ) analysis (Oscar, 2005) was utilized to evaluate predictions made by the Baranyi model from each dynamic temperature profile. Predicted values were subtracted from observed values to generate the prediction error (PE) for each observation with $\log_{10}$ CFU/g units. Positive PE values were considered fail-dangerous and PEs with a negative value were considered fail-safe, while a PE of 0 indicated a perfect prediction (Oscar, 2005). Acceptable prediction zone limits were set between −1.0 and 0.5 log CFU/g (Mishra et al., 2017).

3. Results and discussion

3.1. Primary models

No naturally occurring *B. cereus* spores were found in uninoculated samples. Growth from *B. cereus* spores in cooked rice was not observed at 10 and 49 °C during storage for 28 and 4 days, respectively. The four primary growth models used in the study were fitted to growth data for *B. cereus* collected under isothermal conditions (13–46 °C) in cooked rice. Fig. 1 illustrates a representative trial for each temperature point, displaying observed growth and primary growth model predicted growth. Graphically, each primary growth model fitted the growth data well. Fit behavior differed for each model, dependent on the type of model that it is. For example, the Huang model tends to have a sharp angle at the point of transition between lag and exponential phase, as it attempts to clearly define the two phases (Huang, 2011).

The h_o term is a parameter in the Baranyi model that attempts to account for the physiological state of bacteria during growth (Baranyi and Roberts, 1994). It is assumed that this term is constant for a specific bacterial species in a given growth substrate. Because of this, h_o values were averaged over all isothermal temperature profiles and the model was run again to estimate the other three parameters in the model with the average h_o value, $\overline{h_o}$, fixed (Amézquita et al., 2005; Baranyi and Roberts, 1994; Juneja et al., 2007; Juneja et al., 2018). Values for h_o ranged from 1.75 to 8.49 and the average value was 4.10. This value differs slightly from others in the literature. For *Clostridium perfringens* in cooked ham, an $\overline{h_o}$ of 6.7 was reported (Amézquita et al., 2005), while an $\overline{h_o}$ of 1.7 was reported for *Salmonella* in chicken (Juneja et al., 2007). For *Brochothrix thermosphacta* in a broth medium, an $\overline{h_o}$ of 3.2 was reported (Baranyi et al., 1995). These differences are likely due to the vast differences in the growth media used and microorganism tested.

Goodness of fit statistics for each primary growth model's performance at each isothermal growth temperature are displayed graphically in Supplemental Fig. 1. By all three goodness of fit measures, all four models had acceptable performance (i.e. high R^2, low RMSE/SSE) across all growth temperatures. The modified Gompertz model performed the best of the four models at extreme temperatures (13–19 °C and 40–46 °C), while the Baranyi model performed marginally worse than the other models at higher temperatures (40–46 °C). All models performed well at the intermediate temperatures (22–37 °C). This is likely due to the fact that this range includes the ideal growth temperature for *B. cereus* (El-Arabi and Griffiths, 2013), making its growth more predictable. Due to the acceptable performance of all four primary growth models and the fact that the Baranyi model is well equipped to predict bacterial growth at varying temperatures due to the model's dynamic nature, the Baranyi model was chosen for tertiary modeling. Although the other primary models showed acceptable performance in isothermal conditions, the models are not built for dynamic conditions and thus were not considered for tertiary modeling.

3.2. Secondary models

The results for the estimated parameters of the modified Ratkowsky secondary model are displayed in Table 1. Fig. 2 illustrates the fitted modified Ratkowsky model to observed μ_{max} values for each primary model. The high R^2 values (Table 1; all > 0.90) indicate that the modified Ratkowsky model can be used to effectively estimate maximum specific growth rate for *B. cereus* in cooked rice for all four primary models. According to goodness of fit statistics, the Baranyi model (R^2 = 0.9914, RMSE = 0.08046) was best fitted by the modified Ratkowsky secondary model due to it having the highest R^2 and lowest RMSE values, followed by the other three models. While it was determined that the modified Ratkowsky model fitted the μ_{max} values generated from the Baranyi model the best, the secondary model parameters for the other three primary models are included in the results because these are also very commonly used in predictive microbiology. For example, the modified Gompertz, Baranyi, and Huang models are used in the USDA Pathogen Modeling Program (PMP), ComBase Predictor, and USDA Integrated Pathogen Modeling Program (IPMP), respectively.

The T_{min} and T_{max} values estimated by the modified Ratkowsky model (Table 1) are the theoretical (notational) temperatures at which no growth will occur, not the actual minimum and maximum temperatures at which growth ceases that are observed in the literature (Zwietering et al., 1996). Extrapolated minimum and maximum growth temperatures are commonly lower or higher, respectively, of the actual observed minimum and maximum growth temperatures (McKellar and Delaquis, 2011). In the present study, growth of *B. cereus* in cooked rice only occurred between 13 °C and 46 °C. These temperatures were used as the minimum and maximum temperatures used in the tertiary model to avoid any sort of added fail-safe measure. Predicted T_{min} and T_{max} values for *B. cereus* in cooked rice for the modified Gompertz, Baranyi, and Huang models are similar to those predicted by the same primary growth models for *B. cereus* in cooked beans (Juneja et al., 2018).

The effect of temperature on the lag phase duration (LPD) of *B. cereus* in cooked rice estimated by the four primary growth models was also described using a hyperbolic function. The results of the estimated parameters of the hyperbolic function for each primary model are displayed in Table 2. Fig. 3 illustrates the fitted LPD hyperbolic function to observed LPD values for each primary model. The hyperbolic function fits the LPD from all models well, as indicated by the high R^2 and low RMSE values (Table 2). The Baranyi model LPD was best fitted by the hyperbolic function in regard to R^2 and RMSE statistics.

3.3. Validation using dynamic temperature profiles

The growth ($\log_{10}$ CFU/g) of *B. cereus* in cooked rice during single rate (exponential) and biphasic linear cooling is presented in Table 3. As the single exponential cooling rate (54.5 °C–7.2 °C) decreased (i.e. cooling time increased), bacterial growth increased. In the 21-hour profile, there was about a 4.0 $\log_{10}$ CFU/g increase from pre-cool to post-cool rice. For the six-hour profile, there was only about a 0.32 $\log_{10}$ CFU/g increase from pre-cool to post-cool rice.

Similarly, during biphasic linear cooling, the cooling rate significantly impacted bacterial growth ($\log_{10}$ CFU/g). When the duration of the first step of cooling (54.5 °C–27.0 °C) was 1.5 h, bacterial numbers only increased about 0.32 $\log_{10}$ CFU/g. When the first step of cooling was increased to 6.5 h, the bacterial population increased by about 2 $\log_{10}$ CFU/g from pre-cool to post-cool rice. Because the optimum growth temperature for *B. cereus* is 30–40 °C (El-Arabi and Griffiths, 2013), the longer that foods spend in this range of temperatures, the higher the level of growth of vegetative *B. cereus* cells.

The dynamic Baranyi model (Eqs. (17) and (18)) was used to predict *B. cereus* growth behavior for the single rate (exponential cooling) and biphasic linear cooling experiments. Predictions were compared with observed values and APZ analysis was performed (Fig. 4). For single rate cooling, 23 of the 24 predictions fell within the APZ (−1.0 to 0.5 log CFU/g). For biphasic linear cooling, 26 of the 28 predictions fell within the APZ.

For single rate cooling profiles, Baranyi-predicted and observed bacterial growth behavior is illustrated in Fig. 5. Predicted and observed generation time values for each single rate cooling profile were compared, and accuracy and bias factor statistics were generated. The accuracy factor and bias factor for the single rate cooling validation experiment were 1.05 and 1.02, respectively. Both numbers show good performance of the Baranyi model to predict growth of *B. cereus* during single rate exponential cooling of cooked rice. For bias factor, a range of 0.70–1.15 has been suggested to indicate models that are “acceptable” predictors of bacterial growth (Oscar, 2005; Ross, 1996). This helps to validate the generated dynamic model.

Due to the nature of growth data collection in this experiment, there was a time gap between the last observation collected during exponential phase and first observation during stationary phase for isothermal temperature profiles 25–46 °C (Fig. 1). In theory, this could have presented a bias in the μ_{max} values obtained during the fitting of primary growth models. While this can be seen as an experimental limitation, the dynamic model validation results show that the model still performed acceptably well at predicting the growth of *B. cereus* in cooked rice at varying temperatures. This being said, future experimental approaches should be modified to collect samples throughout the whole exponential phase and beginning of stationary phase to avoid any sort of estimated growth parameter biases.

In conclusion, the Baranyi model performed best in terms of secondary model performance and dynamic temperature model validation. Acceptable prediction zone analysis further confirms its usage under non-isothermal conditions. The presented model will help the food industry determine safe cooling times for cooked rice to prevent outgrowth of *Bacillus cereus* that could cause foodborne illness to consumers. The model will also be useful in predicting *B. cereus* growth in rice in case of temperature deviations. Furthermore, these data will be very useful in developing critical limits for safe cooling of cooked rice. However, it is worth pointing out that the spore production conditions, such as sporulation temperature and medium, alter the germination and outgrowth of the resultant spores. Since this model will only be applicable to cooked rice with no additives, further studies are warranted to determine the fate of *B. cereus* spores during cooling of cooked rice supplemented with various spices, vegetables, meats, etc. and the subsequent development of a predictive model.

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