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# Growth Kinetic Behavior of Toxin-Producing Bacteria on Strawberries under Dynamic Temperature Conditions

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**This study, developed mathematical models to describe the growth kinetics of toxin-producing bacteria on strawberries under dynamic temperature. *Staphylococcus aureus* and *Bacillus cereus* were inoculated on strawberry. The samples were then stored at 4-30°C. *S. aureus* and *B. cereus* were grown on mannitol-salt agar and mannitol egg-yolk polymyxin agar, respectively. The microbial data were fitted to the Baranyi model to calculate the maximum specific growth rate ($\mu_{max}$) and lag-phase duration. The parameters were further analyzed using a polynomial model, and dynamic models were developed to simulate bacterial growth at changing temperature. The bacterial growth gradually decreased at 4 and 10°C, but increased at 15-30°C on strawberries. The secondary model showed that temperature slightly influenced the $\mu_{max}$ values. However, the simulation showed that *S. aureus* and *B. cereus* cell counts were not altered by dynamic temperature condition, i.e., those that exist during commercial distribution of strawberries.The results imply that the initial numbers of *S. aureus* and *B. cereus* on strawberries were not changed during distribution, and thus, an initial contamination level lower than the threshold for enterotoxin production may not cause foodborne illnesses. This result can be used to determine new control measure for *S. aureus* and *B. cereus*.**

**Key words:** Strawberry, Toxin-producing bacteria, Dynamic model.

Strawberries are widely consumed across many countries because of their pleasant taste and health benefits (Kim *et al*., 2006), but they are perishable fruits, susceptible to mechanical injury, desiccation, decay, and physical contact during storage (Vu *et al*., 2011). Strawberries are usually consumed without a decontamination step and further processing. Because of this, their consumption can cause food borne illnesses if they are contaminated with pathogens. Nevertheless, there are a limited number of studies regarding the effect of storage conditions on bacterial growth on strawberries (Nabutaka *et al*., 2007).

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*Staphylococcus aureus* and *Bacillus cereus* are toxin-producing bacteria. *S. aureus* is gram-positive and one of the major food borne pathogens (Asperger and Zangerl, 2002). *S. aureus* cells are usually found in the nails, skin, and hair of 20-30% of the world's population, and the pathogen can be transferred to foods by handling (Van Belkum *et al*., 2009). According to the report by the Korean Ministry of Food and Drug Safety, 4.0% of food borne illnesses were caused by *S. aureus* (MFDS, 2011). Other examples of outbreaks due to strawberries occurred in the USA from 2005 to 2007 (CDC, 2008).

*B. cereus* is a gram-positive, spore-forming, motile, and aerobic bacterium that produces diarrheal and emetic toxin (Granum and Lund, 1997). The diarrheal type disease is caused by complex enterotoxins, which are produced by vegetative *B. cereus* cells in the small intestine,

while the emetic toxin is produced by growing cells in the food (Granum and Lund, 1997). Since *B. cereus* spores can be isolated from soil (Stenfors Arnesen, 2008), they can contaminate strawberries.

Predictive models can be used to quantify bacterial growth and death in accordance with different parameters such as temperature, water activity, humidity, and pH (Baranyi and Roberts, 1994). Primary models predict bacterial growth over time, and secondary models describe the effects of various factors on kinetic parameters (Whiting, 1995). Most predictive models have been designed for constant temperatures, but the conditions for strawberry distribution and storage are dynamic, especially for temperature.

Therefore, the objective of this study was to develop predictive models describing the growth of toxin-producing bacteria on strawberries under changing temperature conditions.

## MATERIALS AND METHODS

### Preparation of inocula

Five strains of *S. aureus* (ATCC13565, ATCC27664, ATCC14458, ATCC23235, and NCCP10826) and five strains of *B. cereus* (KCTC1013, KCTC1014, KCTC1092, KCTC1094, and KCTC3624) were cultured in 10 mL trypticsoy broth (TSB;Bacto, Becton Dickinson, MD, USA) at 35°C for 24h, and 0.1 mL of the culture were subcultured in 10 mL TSB at 35°C for 24h. Cultures of the five strains were mixed for each of the two species, and the mixtures were harvested by centrifugation for 15 min at 1912×*g* and 4°C. To make the inocula, the resulting pellets were washed twice with phosphate buffered saline (PBS, pH 7.4; 0.2 g/L $LKH_2PO_4$, 1.5 g/L $Na_2HPO_4$, 8.0 g/L NaCl, and 0.2 g/L KCl in distilled water), and finally, diluted in PBS to 3-4 log CFU/mL.

Since strawberries may be contaminated by spores rather than vegetative cells of *B. cereus* from the environment, spores were used for the inoculum. Colonies of *B. cereus* on mannitol-salt egg-yolk polymixin-Bagar (MYP;Difco, Becton Dickinson, MD, USA) were inoculated into 10 mL TSB and incubated at 35°C for 24 h. Then, 0.1 mL portions of these cultures were subcultured in 10 mL TSB at 35°C for 24 h, followed by centrifugation (1912 ×*g*, 4°C, 15 min). The resulting pellets were washed with PBS, and serially diluted in PBS to 3-4 log CFU/mL. The 0.1 mL portions of the diluted suspension were surface-spread on to nutrient agar (NA;Difco) supplemented with $MnSO_4$ and $CaCl_2$, each at 0.001%. The plates were incubated at 35°C for 4 days (Ankolekar and Labbé, 2009). Three milliliter of PBS was added directly to the colonies, which were collected using a glass rod, followed by centrifugation for 15 min at 1912×*g* and 4°C (Lee *et al*., 2013). The spore pellets were washed twice with PBS and serially diluted in PBS to 4 log CFU/mL.

### Inoculation of strawberries and quantification of bacteria and pH

Portions (0.1 mL) of *S. aureus* or *B. cereus* inocula were inoculated on the surface of strawberries at approximately 3 logCFU/g. After inoculation, the samples were left for 15 min to allow bacterial cell attachment. The strawberries were placed in polystyrene-foam boxes and covered with plastic film. The box was then stored at 4, 10, 15, 20, 25, and 30°C for up to 96h. The samples were then analyzed every 24 h. Three strawberries (80±15 g) were aseptically transferred to a filter bag (Sample bag, 3M, Korea) containing 80 mL of 0.1% buffered peptone water (BPW; Difco) and the samples were homogenized using a blender (BagMixer, Interscience, France) for 90 s. The homogenates were serially diluted with 9mLBPW, and 0.1 mL portions of the diluents were plated on tryptic soy agar (TSA; Difco), mannitol-salt agar (MSA;BBL, Becton Dickinson, MD, USA), and MYP agar for total bacteria, *S. aureus* and *B. cereus* cell counts, respectively. The plates were then incubated at 35°C for 24 h. The pH values of the homogenates were measured with a digital pH meter (Accument, Denver Instruments, CO, USA).

### Enterotoxin measurement

To measure the enterotoxin, 1 mL of the homogenate that was used for microbial analysis, was examined. The enterotoxin produced by *S. aureus* and *B. cereus* were measured with a Tecra Staph Enterotoxins Visual Immunoassay (3M, Australia) and a BECT-RPLA toxin detection kit (Oxoid, England), respectively, according to the manufacturer's instructions.

### Model development

The experimental data for *S. aureus* and *B. cereus* were fitted to the Baranyi model (Baranyi and Roberts, 1994) to estimate kinetic parameters

such as the maximum specific growth rate ($\mu_{max}$; log CFU/g/h) and the lag phase duration (*LPD*; h) with DMF it (Institute of Food Research,UK). A quadratic polynomial model was then used to describe the effect of temperature on $_{\mu max}$ values as follows;

$$\mu_{max} = a_0 + a_1.T + a_2.T^2 \qquad ...(1)$$

Where $a_i$ is the coefficient, and *T* is the storage temperature (°C). To describe the growth patterns of *S. aureus* and *B. cereus* at changing temperatures, which simulate distribution and storage conditions, the mathematical model defined by Baranyi and Roberts (Baranyi and Roberts, 1994) was used. The dynamic temperature data were collected from three cities during distribution and storage. Changing temperature was recorded by an electronic temperature recorder (Testo 174H, Testo, NJ, USA).

To evaluate the model's performance, *S. aureus* and *B. cereus* were inoculated on strawberries. These samples were exposed to range of temperatures according to the temperature data collected above, and the bacterial populations were then enumerated as described previously. The results were compared to the values predicted by the model.

## RESULTS AND DISCUSSION

During storage, no significant growth of total bacteria was observed ( data not shown). The $\mu_{max}$ value of *S. aureus* indicates that no significant growth of *S. aureus* was observed at any storage temperatures, but *B. cereus* showed a slight increase ingrowth (Table1). The $R^2$ values for the primary models varied from 0.621-0.969 (Table1).

The lack of any significant growth on strawberries may be caused by their low pH. The pH of the samples ranged from 3.5 to 4.5 during storage at 4-30°C for 96 h (data not shown). The optimum pH values for growing *S. aureu* and *B. cereus* are 6-7 and 4.9-9.3, respectively (Gilbert and Kramer, 1981; Tatini, 1973). In addition, the potent antioxidant activity of phenolic compounds in strawberries may also inhibit bacterial growth (Guo *et al*.,2007; Lacombe *et al*., 2010; Nohynek *et al*., 2006).

Since no significant growth was observed, enterotoxins for both bacteria were also absent from measurements (data not shown). *S. aureus* generally produces enterotoxin at 5-6 log CFU/g in foods (FDA, 2012), and therefore we inoculated a high concentration (7 log CFU/g) of *S. aureus* onto the strawberries. However, no enterotoxin production was observed, indicating that *S. aureus* only produces enterotoxin when

**Table1.** Parameters from primary modeling for *Staphylococcus aureus* and *Bacillus cereus* growth on strawberry

| Bacteria | Storage temperature (°C) | $\mu_{max}$ (log CFU/g/h) | $Y_0$ (log CFU/g) | $Y_{max}$ (log CFU/g) | $R^2$ |
|---|---|---|---|---|---|
| *Staphylococcus aureus* | 4 | -0.031 | 2.19 | 2.22 | 0.920 |
| | 10 | -0.017 | 2.28 | 2.49 | 0.750 |
| | 15 | -0.035 | 2.38 | 2.74 | 0.788 |
| | 20 | -0.022 | 2.59 | 2.81 | 0.634 |
| | 25 | -0.031 | 2.82 | 2.51 | 0.621 |
| | 30 | -0.003 | 2.34 | 2.63 | 0.722 |
| *Bacillus cereus* | 4 | -0.006 | 4.00 | 4.03 | 0.621 |
| | 10 | -0.239 | 4.27 | 4.52 | 0.647 |
| | 15 | 0.068 | 3.57 | 3.95 | 0.969 |
| | 20 | 0.053 | 3.57 | 3.92 | 0.823 |
| | 25 | 0.052 | 3.57 | 3.98 | 0.570 |
| | 30 | 0.059 | 3.57 | 3.88 | 0.623 |

$\mu_{max}$: maximum specific growth rate, $Y_0$: lower asymptote, $Y_{max}$: upper asymptote

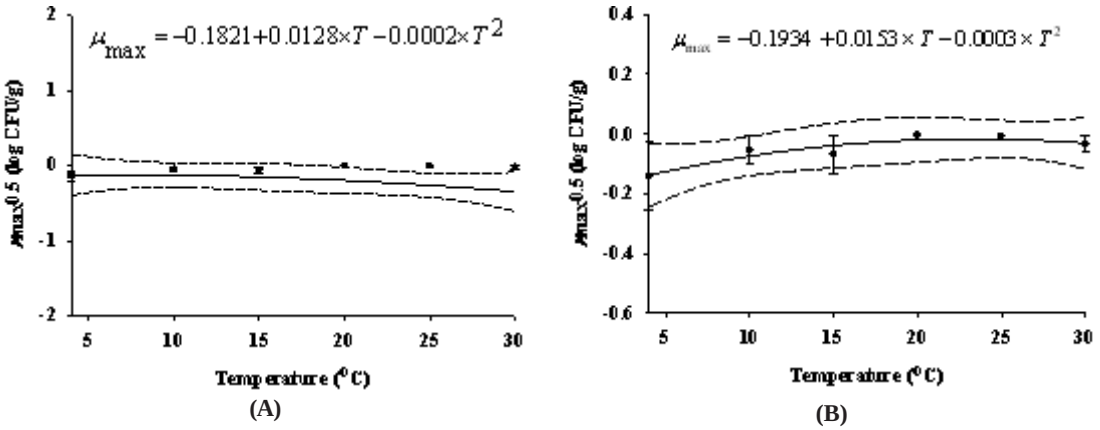


**Fig. 1.** Secondary modeling the $\mu_{max}$ for strawberry, derived from the modified the Baranyi model;• : observed values; : predicted line; — : 95% confidence interval;(A) *Staphylococcus aureus* and (B) *Bacillus cereus*

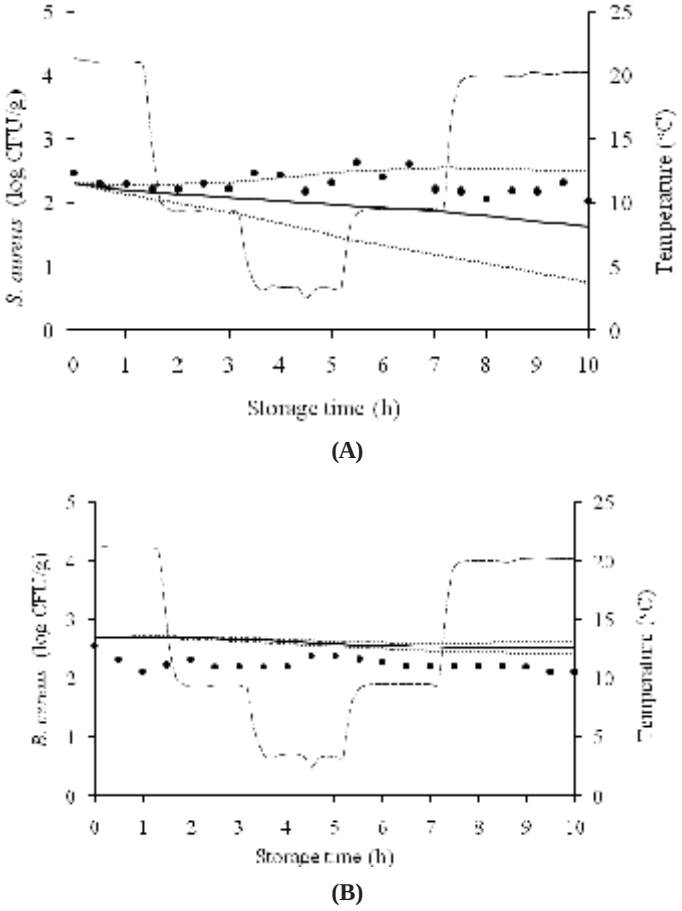


**Fig. 2.** Predicted cell counts of (A) *Staphylococcus aureus* and (B) *Bacillus cereus*on strawberries under changing temperature; •: observed values; : predicted line; : 95% confidence interval; temperature (°C)

the pathogen is proliferating, even at initial cell concentrations of over 6 log CFU/g.

Although no significant bacterial growth was observed, a secondary model was necessary to examine dynamic temperature condition. The effect of storage temperature on $\mu_{max}$ was described by a quadratic polynomial model, and the results showed no temperature effect on $\mu_{max}$ as expected (Fig. 1). The secondary models polynomial models are probably the most common, and the polynomial model can includes the co efficients that have no biological interpretation (Ross and Paw, 2004). In our study, there were no interactions between bacteria and storage temperature, and thus, no biological interpretation was applicable. The polynomial model was then used to develop a secondary model in this study. *LPD* is the period required to overcome an initial hurdle ($h_0$), which requires adaptation work (Munoz-Cuevas *et al*., 2010). In this study, *LPD* was estimated up to 96 h, because no significant growth was observed. The values were then used to calculate $h_0$, which is the product of $\mu_{max}$ and *LPD* and a dimensionless parameter quantifying the initial physiological state (Grijspeerdt, 1999). Under conditions of changing temperature, replicating those found in commercial setting, *S. aureus* cell counts were predicted, and the values were compared with the experimental values. Predicted populations were not changed for either bacterium (Fig. 2). For *S. aureus*, most predicted values were close to those found by experiment, but the experimental values for *B. cereus* were lower than the predicted ones by approximately 0.5 log CFU/g from 0 to 4 h (Fig. 2). This result indicates that our model for dynamic temperature condition may be applicable to simulation of bacterial growth.

In conclusion, the dynamic mathematical models developed here should be useful for evaluation of the risk posed by enterotoxin-producing bacteria such as *S. aureus* and *B. cereus* on strawberries because the models calculate consistently the impact of different steps related to the distribution and retailing of strawberry on microbial dynamics (Bernaerts *et al*., 2004). Moreover, the results from this study could be used to determine new control measures for *S. aureus* and *B. cereus*.

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