Avens Publishing Group
J Bioanal Biostat
August 2015 Volume:1, Issue:1

Open Access Research Article

Journal of
Bioanalysis & Biostatistics

A Dynamic Model for Toxin-Producing Bacteria on Tomatoes

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Submission: 31 July, 2015
Accepted: 14 August, 2015
Published: 20 August, 2015

Keywords: *Staphylococcus aureus*, *Bacillus cereus*, tomato, dynamic model

Abstract

We developed a dynamic model to determine the growth patterns of toxin-producing bacteria on tomatoes during their storage and distribution. Dynamic temperature profiles were also recorded while tomatoes were stored, and during their distribution to three cities. Tomatoes were inoculated with *Staphylococcus aureus* and *Bacillus cereus*, and the bacterial cell counts were then enumerated. The Baranyi model was fitted to the cell counts to calculate death rate and lag phase duration (LPD). These parameters were then fitted to a second order polynomial in the inverse temperature to evaluate the effects of temperature fluctuations. The developed model was validated with observed values, and the root mean square error (RMSE) was calculated. A dynamic model was then developed with the results of the secondary model, and *S. aureus* growth patterns were simulated. Death rates of *S. aureus* ranged from −0.157 to −0.024 log CFU/g/h, depending on the storage temperature. No difference in the death rates was observed among storage temperatures, and LPD was not observed for all storage temperatures. The model performance was appropriate with 0.55 of RMSE. *B. cereus* cell counts decreased rapidly and thus, predictive model was not developed. In conclusion, *S. aureus* cell counts on tomatoes may not be changed at ≥10 °C, but *B. cereus* cannot survive on the produce. In addition, the developed model should be appropriated to describe the fate of *S. aureus* on tomato.

Introduction

Tomatoes are widely consumed across the world [1]. When tomatoes are left at room temperature for a prolonged period, there is an increased risk of their contamination with pathogens [2]. There were also reports related to the identification of pathogenic bacteria on tomatoes [3]. Therefore microbiological management is necessary, especially for toxin-producing bacteria.

The bacteria *Staphylococcus aureus* and *Bacillus cereus* are associated with major food-borne illnesses [4]. Gram-positive *S. aureus* is known to produce harmful toxins [5], and can grow at pH 4.5–9.3 with an Aw in excess of 0.83, and a sodium chloride concentration up to 20% [6]. Therefore, *S. aureus* is prevalent in a wide variety of foods [7]. In addition, *S. aureus* on worker's hands might be transferred to tomatoes during harvesting and packaging. Gram-positive *B. cereus* is a spore-forming, toxin-producing bacterium [8]. This pathogen is widely distributed in nature and found in various foods. It is also known to cause food-borne illnesses in humans associated with vomiting and diarrhea [9]. The spread of *B. cereus* is facilitated by spores, while the toxin it produces is responsible for illness. *B. cereus* can be grown at pH 4.3–9.3 and even in low moisture such as 0.91 of Aw [10,11]. Also, vegetative cells and spores of *B. cereus* can be cross-contaminated to tomatoes from soil.

Predictive microbiology is useful in studying and predicting the growth and death of microorganisms by using mathematical equations [12]. The purpose of predictive microbiology is to ensure food safety in advance, allowing stakeholders to address all possible risk factors in advance [13]. Most predictive models have been developed for constant temperatures, with environmental changes, such as temperature and humidity, during food distribution not usually considered. The development of a dynamic model using primary and secondary models is considered appropriate to describe the fate of bacteria in changing environments.

The objective of our study was to develop a dynamic model of toxin-producing *S. aureus* and *B. cereus* on tomatoes and predict their behavior.

Materials and Methods

Preparation of inocula

Considering strain variation, five-strain mixtures for *S. aureus* and *B. cereus* were prepared as inocula as follows. Five *S. aureus* strains [ATCC13565 (SEA; staphylococcal enterotoxin A), ATCC14458 (SEB), ATCC23235 (SED), ATCC27664 (SEE), and NCCP10826 (SEC)] and five *B. cereus* strains (KCTC1013, KCTC1014, KCTC1092, KCTC1094, and KCTC3624) were cultured in 10 ml of tryptic soy broth (TSB; Becton, Dickinson and Company, Sparks, MD, USA) at 35 °C for 24 h. Aliquots (0.1 ml) of cultures were transferred into 10 ml fresh TSB for subculture at 35 °C for 24 h. For each bacterial species, the cultures of the five strains were mixed. Each mixture was then centrifuged (1,912 × *g*, 15 min, 4 °C), and the cell pellets washed twice with phosphate-buffered saline (PBS; 0.2 g of KH_2PO_4, 1.5 g of Na_2HPO_4, 8.0 g of NaCl, 0.2 g of KCl, 1 l of distilled water, pH 7.4). Cell suspensions of the two bacterial species were diluted with PBS to 6–7 log CFU/ml.

ISSN: 2641-8681

Inoculation and enumeration of bacterial cell counts

The 0.1 ml aliquots of *S. aureus* or *B. cereus* were inoculated on the surface of intact tomatoes at approximately 5 log CFU/g, and left for 15 min to allow for the attachment of bacterial cells. Following inoculation with *S. aureus*, tomatoes were placed in plastic containers and stored at 4, 10, 15, 20, 25, and 30 °C for up to 7 days, with samples analyzed every 24 h. Tomatoes inoculated with *B. cereus* were stored at 30 °C for up to 3 h. Every tomato (180 ± 20 g) was aseptically transferred to a filter bag (3M™, Seoul, Korea) containing 100 ml of 0.1% buffered peptone water (BPW; Becton, Dickinson and Company, Sparks) and homogenized for 30 s (BagMixer®, Interscience, St. Nom, France). Homogenates were serially diluted with 9 ml BPW, and 0.1 ml of the diluents was plated on tryptic soy agar (TSA; Becton, Dickinson and Company) for total bacteria, mannitol salt agar (MSA; Becton, Dickinson and Company) for *S. aureus*, and mannitol-salt egg-yolk polymyxin agar (MYP; Becton, Dickinson and Company) for *B. cereus*. Plates were incubated at 35 °C for 24 h. After microbial analysis, the pH values of the homogenates were measured with a digital pH meter (Accumet®, Denver Instruments, Arvada, CO, USA).

Enterotoxin measurement

Because 78% of *S. aureus* intoxication is caused by SEA [14], SEA production was measured. To measure SEA production by *S. aureus* on tomatoes during storage, 1 ml aliquots of the homogenates used for the quantitation of bacterial populations at 25 and 30 °C were analyzed using a Tecra Staph Enterotoxins Visual Immunoassay (3M™, North Ryde, NSW, Australia) according to the manufacturer's instructions. Since *B. cereus* cell counts decreased rapidly on tomatoes, the enterotoxin production was not measured.

Model development

The Baranyi model [15] was fitted to the *S. aureus* cell count data to estimate kinetic parameters such as death rate (log CFU/g/h) which is defined as μ_{max} in a growth curve, and lag phase duration (LPD; h), and initial cell concentration (N_0; log CFU/g) and the lowest cell concentration in a death curve (N_{max}; log CFU/g) with DMFit (http://www.ifr.ac.uk/safety/dmfit/; Institute of Food Research, Norwich, UK) which is an Excel add-in to fit curves with the Baranyi model [15] as follow:

$$N_t = N_0 + \mu_{max} \times A_t - \ln\left[1 + \frac{\exp(\mu_{max} \times A_t) - 1}{\exp(N_{max} - N_0)}\right] \quad (1)$$

Where N_t is the *S. aureus* cell counts at time *t*, and N_0 and N_{max} is the initial and the lowest *S. aureus* cell counts in a death curve, respectively. A_t is the adjustment function, which denotes physiological status of *S. aureus* cell to define the LPD [15].

We use a second order polynomial in the inverse temperature to describe the effect of temperature on death rate values as follows:

$$[Death\ rate = a_0 + a_1 / T + a_2 / T^2] \quad (2)$$

Where a_i is the coefficient and T is the storage temperature (°C). To describe the growth patterns of *S. aureus* under changing temperatures which also simulates the conditions of tomato distribution, the equation (*1*) was used.

Validation

Given that *S. aureus* growth data on tomatoes was not available in the published literature, additional experiments at 12, 18, and 23 °C were conducted. The observed data were then compared with predicted *S. aureus* cell counts (log CFU/g), which were calculated by developed models. The model performance was evaluated using root mean square error (RMSE) [16]:

[RMSE = square root [Σ (observed values – predicted values)^2/n]] (3)

Where *n* represents the number of data points.

Changes in temperature during the distribution of tomatoes to three cities were recorded using a Testo 174H electronic temperature recorder (Testo, Sparta, NJ, USA). To evaluate the performance of the model when temperatures were changing, *S. aureus* population data were collected as the temperature fluctuated and compared with predicted *S. aureus* populations as simulated by a dynamic model.

Results and Discussion

During storage, the growth of *S. aureus* was not observed at all investigated temperatures. The death rate of *S. aureus* ranged from −0.157 to −0.024 log CFU/g/h, and was dependent on the storage temperature (Table 1). At 4 °C, the decrease in *S. aureus* cell counts was obvious with −0.157 log CFU/g/h of death rate. However, at 10–30 °C there was only a minimal decrease in *S. aureus* cell counts (Table 1). The correlation coefficients (R^2) for the primary models varied from 0.733–0.811 (Table 1). In addition, no SEA production by *S. aureus* was observed (data not shown). Cell counts of *B. cereus* rapidly decreased below detection limit (0.5 log CFU/g/ml), thus a primary model was not developed. The lack of significant bacterial growth on tomatoes could be due to evaporation occurring at the surface of tomatoes during storage [17], or their waxy surface, which likely protects them from bacterial penetration [18].

Our results indicate that even if *S. aureus* contaminates a tomato, the pathogen did not grow, while *B. cereus* is quickly inactivated. Although no significant *S. aureus* growth was observed at all storage temperatures, a secondary model was necessary for the development of a dynamic model. The effect of temperature on death rate could be described by an inverse second order polynomial model. Our results showed that temperature had no significant effect on death rate (Figure 1). To evaluate the performance of the models, *S. aureus* cell counts

Table 1: The parameters calculated by the Baranyi model for *Staphylococcus aureus* growth inoculated on tomato during storage (Baranyi and Roberts [15]).

Storage temperature (°C)	Death rate (log CFU/g/h)	N_0 (log CFU/g)	N_{max} (log CFU/g)	R^2
4	-0.157±0.161	4.10±0.36	2.00±0.36	0.733
10	-0.050±0.013	4.43±0.38	2.05±0.31	0.764
15	-0.029±0.021	4.03±0.14	2.03±0.65	0.779
20	-0.024±0.012	3.97±0.44	2.01±0.29	0.811
25	-0.085±0.101	3.23±0.34	0.89±0.43	0.782
30	-0.027±0.020	3.56±0.38	1.32±0.36	0.780

N_0: Initial cell concentration.
N_{max}: Maximum cell concentration.

ISSN: 2641-8681

at 12, 18, and 23 °C were conducted but not used in the development of the model. The observed *S. aureus* cell counts were compared with those predicted by the combined primary and secondary models at the given temperatures. To evaluate model performance, many researchers have used accuracy (A) and bias (B) factors. However, it has been pointed out that A and B factors have significant limitations and can result in the incorrect evaluation of model performance [19]. In the current study, we calculated RMSE to evaluate performance. The RMSE values were around 0.55, indicating that the developed model was appropriate. For fluctuating temperatures, *S. aureus* cell counts were predicted and the values compared with experimental values. The observed *S. aureus* cell counts were lower than the predicted values by approximately 1 log CFU/g (Figure 2). This result indicates that our model slightly overestimates under dynamic temperature conditions. A predictive model for *B. cereus* was not developed because the bacterial cell counts were below detection limit after 3 h (Figure 3).

In conclusion, *S. aureus* cell counts on tomatoes were decreased slightly during storage at ≥10 °C, while *B. cereus* was not able to survive on tomatoes. In addition, the developed primary and secondary models should be useful in predicting the behavior of *S. aureus* on tomatoes.

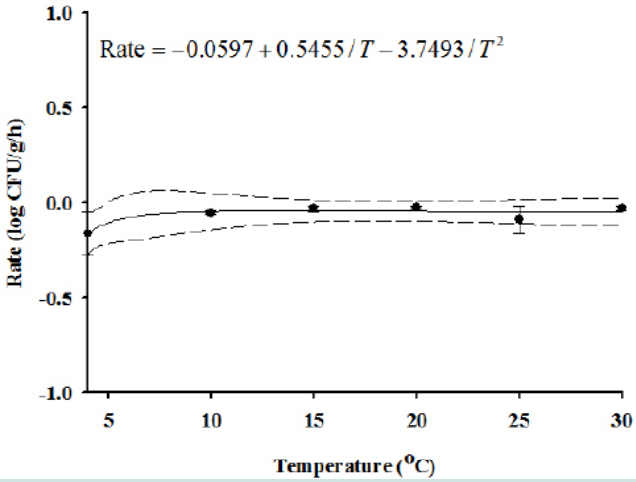

Figure 1: Secondary modeling for the death rates derived from the Baranyi model for the tomato inoculated with *Staphylococcus aureus*; ---: 95% confidence interval.

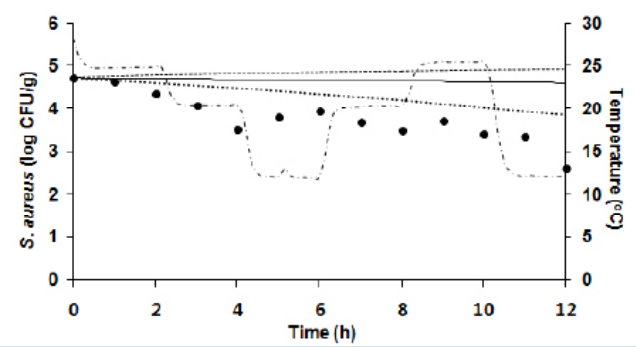

Figure 2: Predicted cell counts of *Staphylococcus aureus* on tomato under changing temperature; •: observed values; —: predicted line; ---: 95% confidence interval; -·-·: temperature (°C).

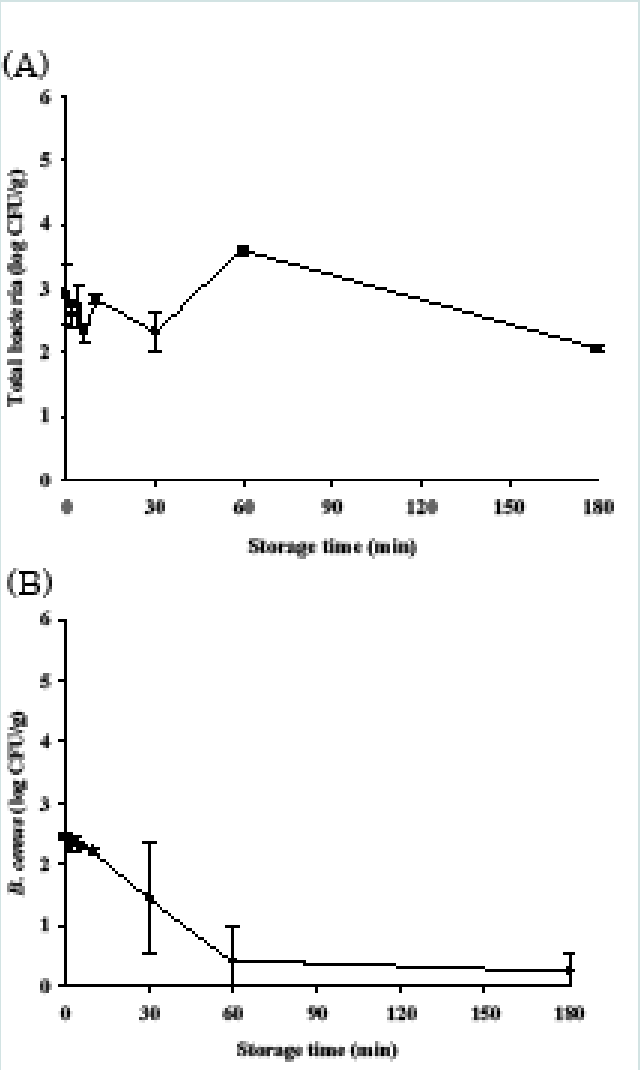

Figure 3: Total bacterial populations (A) and *Bacillus cereus* cells (B) on tomato during storage at 30 °C for up to 180 min.

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ISSN: 2641-8681

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Acknowledgements

This work was carried out with the support of the "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ009404)" through the Rural Development Administration of the Republic of Korea.