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ARTICLE

Development of Kinetic Models Describing Kinetic Behavior of *Bacillus cereus* and *Staphylococcus aureus* in Milk

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Abstract

This study developed predictive models to evaluate the kinetic behaviors of *Bacillus cereus* and *Staphylococcus aureus* in milk during storage at various temperatures. *B. cereus* and *S. aureus* (3 Log CFU/mL) were inoculated into milk and stored at 10°C, 15°C, 20°C, and 30°C, as well as 5°C, 15°C, 25°C, and 35°C, respectively, while bacterial populations were enumerated. The growth data were fitted to the modified Gompertz model to estimate kinetic parameters, including the maximum specific growth rate (μ_{max}; Log CFU/[mL·h]), lag phase duration (LPD; h), lower asymptote (N_0; Log CFU/mL), and upper asymptote (N_{max}; Log CFU/mL). To describe the kinetic behavior of *B. cereus* and *S. aureus*, the parameters were fitted to the square root model as a function of storage temperature. Finally, the developed models were validated with the observed data, and Bias (*B*) and Accuracy (*A*) factors were calculated. Cell counts of both bacteria increased with storage time. Primary modeling yielded the following parameters; μ_{max}: 0.14-0.75 and 0.06-0.51 Log CFU/mL/h; LPD: 1.78-14.03 and 0.00-1.44 h, N_0: 3.10-3.37 and 2.09-3.07 Log CFU/mL, and N_{max}: 7.59-8.87 and 8.60-9.32 Log CFU/mL for *B. cereus* and *S. aureus*, respectively. Secondary modeling yielded a determination of coefficient (R^2) of 0.926–0.996. *B* factors were 1.20 and 0.94, and *A* factors were 1.16 and 1.08 for *B. cereus* and *S. aureus*, respectively. Thus, the mathematical models developed here should be useful in describing the kinetic behaviors of *B. cereus* and *S. aureus* in milk during storage.

Key words: *Bacillus cereus*, *Staphylococcus aureus*, milk, modified Gompertz model

Introduction

Milk is not only well-known nutritious foodstuff for humans but also an excellent matrix that facilitates growth of many types of microbes. For this reason, milk has received much attention, because it may serve as a vehicle for disease agents (Klein, 1901). In fact, raw milk often contains microorganisms that may cause food-borne diseases (Adesiyun *et al*., 1995; Headrick *et al*., 1998; Steele *et al*., 1997). Therefore, in most countries, restrictions and legislation regulating the marketing of unpasteurized milk have been introduced to minimize health risks associated with milk products (European Commission, 2000). However, these do not ensure the safety and quality of dairy products, because diseases associated with milk could occur because of insufficient pasteurization and/or cross-contamination (Altekruse *et al*., 1998; da Silva *et al*., 1998; Gran *et al*., 2003). Moreover, even though the heating process is sufficient to kill vegetative microorganisms, this treatment may be inadequate to remove microbial spores and toxins, such as *Bacillus cereus* and *Staphylococcus aureus* (Rall *et al.*, 2008; Zwietering *et al.*, 1996). Therefore, attention should also be focused on the management of dairy products after pasteurization. Therefore, attention should also be focused on the management of dairy products after pasteurization.

Microbiological challenge tests have been used for determining food safety related to spoilage and pathogenic microorganisms. Simulation of challenge tests should consider the effect of environmental conditions on food, including temperature, pH, and water activity, that could affect the growth and proliferation of microorganisms (Roberts, 1997). Even though challenge tests have been thought to provide general assurance of food safety in the supply chain (Baranyi and Roberts, 1995; Noter-

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mans and in't Veld, 1994; Roberts, 1997), they have limitations: these tests are expensive, labor intensive, time consuming, and non-cumulative as a research tool (McDonald and Sun, 1999). Because of the limitations of challenge tests and the desire for safe, wholesome foods in the supply chain, predictive microbiology has been developed as a relatively new discipline (McMeekin *et al*., 1993).

The objective of this study was to develop mathematical models to describe the kinetic behaviors of *B. cereus* and *S. aureus* in milk under various storage conditions, which could affect their growth and proliferation.

Materials and Methods

Bacterial strains and sample preparation

Milk was purchased from a supermarket in Seoul, Korea. The milk was transported to the laboratory at low temperatures (< 7°C), stored at 4°C, and analyzed within 24 h. The inoculums consisted of *B. cereus* KCCM40935 and *S. aureus* KCCM12193, obtained from the Korea Culture Center of Microorganisms (KCCM, Korea). Each strain was pre-cultured in 10 mL of tryptic soy broth (TSB, Difco, USA) at 35°C at 24 h. Diluted cell suspensions that had been pre-cultured were added to milk at a volume ratio of 1:100 mL, resulting in initial inoculums of approximately 3 Log colony-forming units (CFU)/mL. Samples were then stored at 10°C, 15°C, 20°C, or 30°C for 2 d (48 h) for *B. cereus*, and at 5°C, 15°C, 25°C, or 35°C for 7 d (168 h) for *S. aureus*, and the samples were taken for microbial growth measurements at regular intervals throughout the storage period.

Microbial analysis

To quantify the pathogenic bacteria in milk, *B. cereus* and *S. aureus* were enumerated throughout the storage period (7 d) at different temperatures (*B. cereus*: 10°C, 15°C, 20°C, or 30°C; *S. aureus*: 5°C, 15°C, 25°C, or 35°C). Preparation and microbial analyses were based on standard methods described in the FDA Bacteriological Analytical Manual (FDA, 2010). For each sampling, 10 mL of milk was aseptically transferred into a sterile bottle, and 90 mL of sterile 0.1% peptone water was added. The sample was homogenized for 1 min, and aliquots were plated out directly, or as 10-fold dilutions in 0.1% peptone water. After serial dilution of each sample in sterile peptone water, 0.1 mL aliquot of each sample was separately plated onto each of 2 duplicate agar plates. Viable cells were evaluated by incubating inoculated tryptic soy agar (TSA, Difco, USA) at 35±2°C for 24± 2 h. After incubation, plates with 30–300 colonies were selected for counting. All analyses were performed 3 times, with 2 samples used for each replication, and counts were expressed as Log CFU per milliliter (Log CFU/mL).

Primary model development

Microbiological data (Log CFU/ml) of *S. aureus* and *B. cereus* were fitted to the modified Gompertz model (Gibson *et al*., 1987). The modified Gompertz model is expressed as

$$N_t = A + C \times exp\{-\exp[-B(t-M)]\} \quad (1)$$

where N_t is the bacterial cell number at time t(h), A is the initial cell number of the pathogens, C is the difference between the upper asymptotic line of the growth curve and the lower asymptotic line, B is the relative growth rate at time M, and M is the time at which the growth rate is maximum (h). Maximum specific growth rate (μ_{max}; Log CFU/ml/h), lag phage duration (LPD; h), and N_{max} (Log CFU/mL) were calculated by following equations

$$\mu_{max} = \frac{BC}{e} \quad (2)$$

where e is 2.7182, and

$$LPD = M - \frac{1}{B} \quad (3)$$

$$N_{max} = A + C \quad (4)$$

Secondary model development

For *S. aureus*, μ_{max} and LPD were fitted to the square root model and to a polynomial equation, respectively. The square root model is

$$\sqrt{\mu_{max}} = a_\mu (T - T_{min}) \quad (5)$$

where a_μ is the slope of the regression line for μ_{max}, and T_{min} is the theoretical minimum temperature (°C). The polynomial equation is

$$\sqrt{\frac{1}{LPD}} = a \times T^2 + b \times T + c \quad (6)$$

where a, b, and c are regression parameters, and T is the temperature (°C).

For *B. cereus*, μ_{max} and LPD were fitted to the square root model, and the power model was used for N_{max}. As the μ_{max} of *S. aureus*, square root model was used for

$\sqrt{\mu_{max}}$ of *B. cereus*, and the square root model for LPD is

$$\sqrt{\frac{1}{LPD}} = a_{LPD}(T - T_{min}) \quad (7)$$

where a_{LPD} is the slope of the regression line for LPD. The power model equation is

$$\sqrt{N_{max}} = a \times T^{b} \quad (8)$$

where a and b are regression parameters, and T is the temperature (°C).

Model validation

To validate the developed models, observed bacterial populations were compared to the predicted bacterial populations of the pathogen calculated from the simulation using the developed model (Jung and Lee, 2010; Medved'ová *et al*., 2009). The bias (B) and accuracy (A) factors were calculated as follows (Ross, 1996; Tamplin *et al*., 2005; Zhao *et al*., 2001). The B factor shows whether the models over- or under-predict.

$$B \text{ factor} = 10^{[\Sigma \log(\text{predicted value/observed value})/n]} \quad (9)$$

The A factor describes how close the predicted values are to the observed values,

$$A \text{ factor} = 10^{[\Sigma |\log(\text{predicted value/observed value})|/n]} \quad (10)$$

where n is the number of observed values. The value for perfect agreement between predicted values and observed values is 1 for both factors.

Results and Discussion

Estimation of kinetic parameters

During the storage of milk at 10°C, 15°C, 20°C and 30°C for *B. cereus*, and 5°C, 15°C, 25°C and 35°C for *S. aureus*, growths of these pathogens were observed at temperatures exceeding 15°C. Hence, the growth data at 15°C, 25°C, and 35°C for *S. aureus* were fitted to the modified Gompertz model; similarly, growth data of *B. cereus* at 15°C, 20°C, and 30°C were analyzed using this model. In kinetic parameters for *S. aureus*, the differences of LPDs among storage temperatures were minimal, and μ_{max} values increased up to 0.51 Log CFU/mL/h as the storage temperature increased (Table 1). No effect of storage temperatures on N_0 values was observed, and thus no secondary model for N_0 was developed (Table 1). For *B. cereus*, longer LPD was observed at lower storage temperatures, and μ_{max} values were also markedly increased up to 0.75 Log CFU/mL/h (Table 2). As for *S. aureus*, N_0 values were not influenced by storage temperature, and thus, a secondary model was not developed for N_0 (Table 2). No increase of N_{max} with storage temperature was observed in *S. aureus*, but a correlation between N_{max} and storage temperature was observed in *B. cereus*, and thus N_{max} values were used for secondary modeling only for *B. cereus*. Because R^2 values were greater than 0.926, regardless of storage temperature, and the predicted lines passed through most observed data (Tables 1 and 2, and Figs. 1 and 2), fitting of the growth data to the modified Gompertz model was considered acceptable.

Lag, exponential, and stationary phases in the bacterial growth curve could be influenced by food-related factors and/or environmental conditions (Medved'ová *et al*., 2009). Especially, Baranyi and Roberts (1995) demonstrated that the LPD is a period of adaptation to the environment while the intracellular conditions change. Dens *et al*. (2005) also suggested that medium, temperature, and physiological state of the cells were closely related to LPD. However, in our study, the increase in LPD by storage temperature was observed in *B. cereus* not in *S. aureus* (Tables 1 and 2). This finding indicates that LPD of *S. aureus* in milk may not be affected by storage tem-

Table 1. Kinetic parameters calculated by the Gompertz model for *Staphylococcus aureus* in milk

Temperature (°C)	LPD (h)	μ_{max} (Log CFU/mL/h)	N_0 (Log CFU/mL)	N_{max} (Log CFU/mL)	R^2
15	0.00±0.00	0.06±0.00	2.58±0.30	9.32±0.04	0.966–0.969
25	1.17±2.02	0.33±0.15	2.09±0.56	8.67±0.80	0.926–0.990
35	1.44±0.16	0.51±0.00	3.07±0.09	8.60±0.02	0.945–0.948

LPD: lag phase duration, μ_{max}: maximum specific growth rate, N_0: lower asymptote, N_{max}: upper asymptote

Table 2. Kinetic parameters calculated by the Gompertz model for *Bacillus cereus* in milk

Temperature (°C)	LPD (h)	μ_{max} (Log CFU/mL/h)	N_0 (Log CFU/mL)	N_{max} (Log CFU/mL)	R^2
15	14.03±2.10	0.14±0.00	3.10±0.08	7.59±0.09	0.985–0.993
20	5.35±0.38	0.35±0.03	3.37±0.04	8.70±0.03	0.977–0.994
30	1.78±0.53	0.75±0.11	3.15±0.27	8.87±0.06	0.974–0.996

LPD: lag phase duration, μ_{max}: maximum specific growth rate, N_0: lower asymptote, N_{max}: upper asymptote

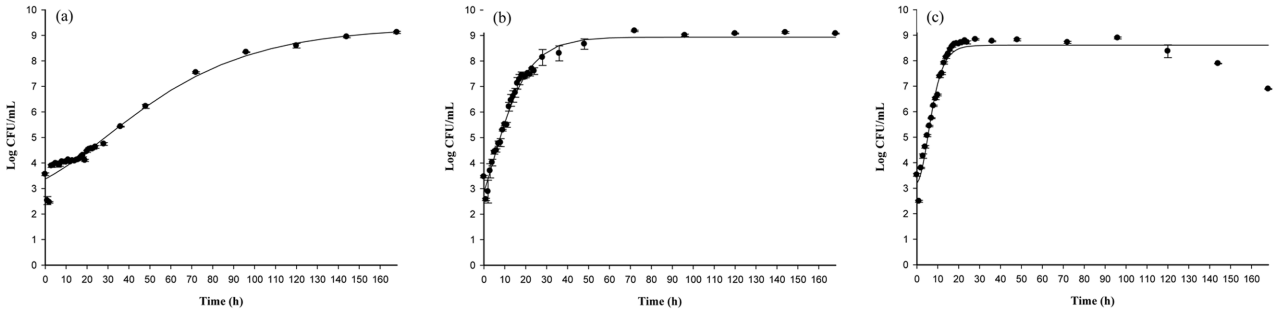

Fig. 1. Observed growth (symbol) and predicted growth (line) of *Staphylococcus aureus* in milk at 15°C (a), 25°C (b), and 35°C (c) for 168 h; the predicted line was produced by primary modeling with the Gompertz model.

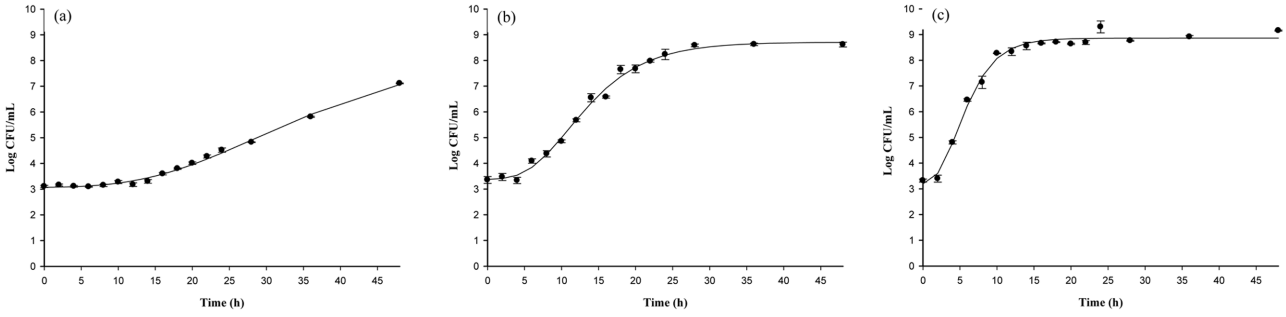

Fig. 2. Observed growth (symbol) and predicted growth (line) of *Bacillus cereus* in milk at 15°C (a), 20°C (b), and 30°C (c) for 48 h; the predicted line was produced by primary modeling with the Gompertz model.

perature.

Secondary modeling

To describe the kinetic behavior of *S. aureus* and *B. cereus*, the kinetic parameters, which were influenced by storage temperatures, were fitted to secondary models. Thus, although a secondary model for N_{max} of *S. aureus* in milk was not developed, secondary models for μ_{max}, LPD, and N_{max} of *B. cereus* were developed as follows:

$$\sqrt{\mu_{max}} = 0.0331(T-3.4108)$$

$$\sqrt{\frac{1}{LPD}} = 0.0329(T-6.8632)$$

$$\sqrt{N_{max}} = 2.0333 \times T^{0.1153}$$

In addition, secondary models describing the kinetic behavior of *S. aureus* in milk were developed as follows:

$$\sqrt{\mu_{max}} = 0.0231(T-2.554113)$$

$$\sqrt{\frac{1}{LPD}} = 0.0024029T^2-0.0783T+0.6342$$

The secondary models showed that the theoretical minimum temperatures for *B. cereus* and *S. aureus* growth were 3.42°C and 2.55°C in milk, respectively. A linear

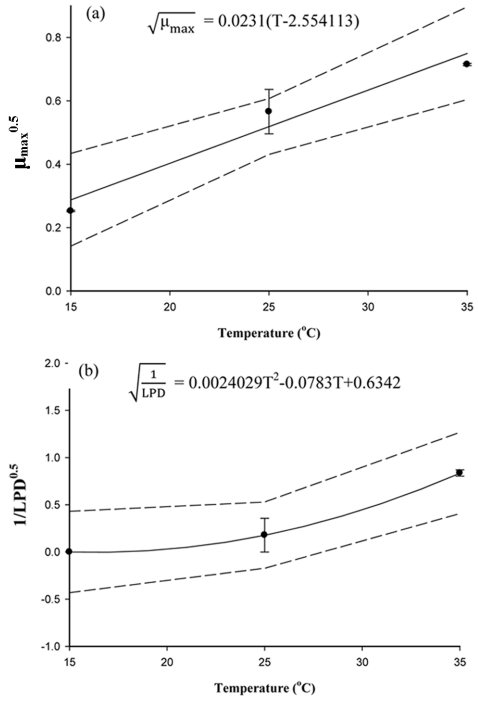

Fig. 3. The square root plot of μ_{max} (a) and the polynomial plot of LPD (b) of *Staphylococcus aureus* in milk as a function of temperature. Symbols represent observed values; lines indicate predicted values according to the square root and the polynomial model; dashed lines indicate lower and upper 95% confidence intervals of the models.

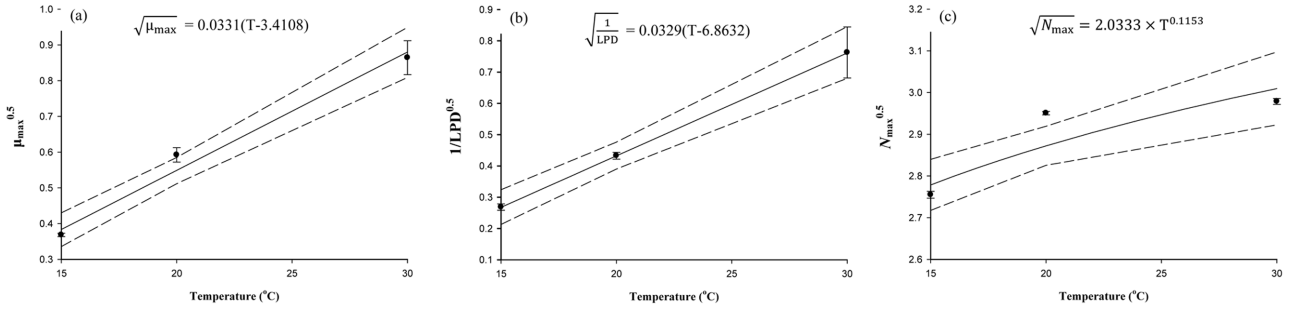

Fig. 4. The square root plot of μ_{max} (a) and LPD (b), and the power plot of N_{max} (c) of *Bacillus cereus* in milk as a function of temperature. Symbols represent observed values; lines indicate predicted values according to the square root and the polynomial model; dashed lines indicate lower and upper 95% confidence intervals of the models.

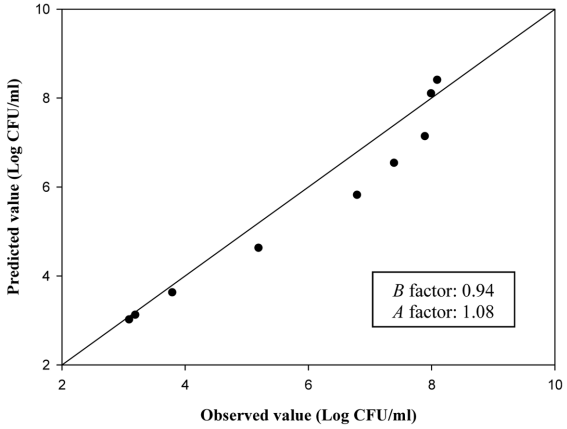

Fig. 5. Plots of observed *Staphylococcus aureus* cell counts against predicted *Staphylococcus aureus* cell counts in milk, after storage at 30°C.

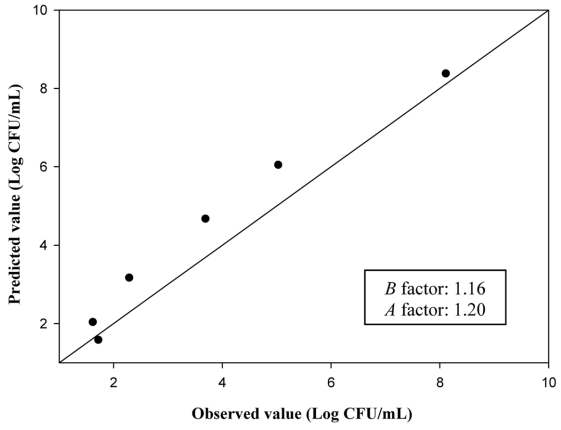

Fig. 6. Plots of observed *Bacillus cereus* cell counts against predicted *Bacillus cereus* cell counts in milk, after storage at 25°C.

relationship between LPD and storage temperature was observed only for *B. cereus* in milk.

The T_{min} for *B. cereus* was in the range found in another study, viz., 9.15°C and 0.04°C in nutrient broth (Nauta *et al*., 2003). The variances of T_{min} values for *B. cereus*, including the T_{min} found in our study, may be caused by strain variation. For T_{min} of *S. aureus*, other studies have shown that the lowest *S. aureus* growth temperature was 6.5-7.0°C (Asperger and Zangerl, 2003; Baird-Parker, 2000; Halpin-Dohnalek and Marth, 1989; Jay, 2000).

Medved'ová *et al.* (2009) evaluated the T_{min} values of 3 *S. aureus* strains in milk, and showed strain variation of T_{min} values (4.89-7.02°C), which are slightly higher than the T_{min} calculated in our study. Thus, the lower T_{min} in our study may be caused by strain variation of *S. aureus*.

Validation

To validate model performance, the data from the studies by Jung and Lee (2010) and Medved'ová *et al*. (2009) were used as observed data. The observed data were compared with the predicted bacterial populations of *B. cereus* and *S. aureus* by using our model simulation, predicted values were plotted against observed values, and *B* and *A* factors were calculated. For *B. cereus*, the *B* and *A* factors were 0.94 and 1.08, respectively (Fig. 5), whereas those for *S. aureus* were 1.16 and 1.2, respectively (Fig. 6). Perfect agreement between predicted and observed values should be 1 for both *B* and *A* factors (Ross *et al*., 2000). If the *B* factor exceeds 1, the model prediction is more than the true value, but if the *B* factor is less than 1, the predicted value is lower than the true value (Ross *et al*., 2000). For instance, the predictive model for *B. cereus* on average under-predicted by 6%, whereas the model for *S. aureus* over-predicted by 16% in our study. Ross *et al.* (2000) suggested that a model yielding a *B* factor of 0.7-0.9 or 1.06-1.15 is considered acceptable, whereas a model yielding a *B* factor of < 0.7 or > 1.5 is considered unacceptable. The *A* factor is a measure of average deviation, and this factor should be greater than 1; the higher this value, the less accurate the prediction (Ross, 1996; Yoon *et al*., 2009). The *A* factors (1.08-1.2) in our study may suggest that the prediction by

the developed model is accurate. Taken together, the predictive models developed in our study could be considered as having good predictive value.

In conclusion, the mathematical models developed here should be useful for describing the kinetic behaviors of *B. cereus* and *S. aureus* in milk during storage at various temperatures.

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References

1. Adesiyun, A. A., Webb, L., and Rahaman, S. (1995) Microbiological quality of raw cow's milk at collection centers in Trinidad. *J. Food Protect*. **58**, 139-146.
2. Altekruse, S. F., Timbo, B. B., Mowbray, J. C., Bean, N. H., and Potter, M. E. (1998) Cheese-associated outbreaks of human illness in the United States, 1973 to 1992: Sanitary manufacturing practices protect consumers. *J. Food Protect*. **61**, 1405-1407.
3. Asperger, H. and Zangerl, P. (2002) *Staphylococcus aureus.* In: Encyclopaedia of dairy sciences. Roginski, H., Guquay, J. W., and Fox, P. F. (eds) Academic Press, San Diego, pp. 2563-2569.
4. Baird-Parker, T. C. (2000) *Staphylococcus aureus*. In: The microbiological safety and quality of food. Lund, B., Baird-Parker, A. C., and Gould, G. W. (eds) Aspen Publishers, Inc., Gaithersburg, pp. 1317-1330.
5. Baranyi, J. and Roberts, T. A. (1995) Mathematics of predictive food microbiology. *Int. J. Food Microbiol*. **26**, 199-218.
6. da Silva, M. C., Hofer, E., and Tibana, A. (1998) Incidence of *Listeria monocytogenes* in cheese produced in Rio de Janeiro, Brazil. *J. Food Protect*. **61**, 354-356.
7. Dens, E. J., Bernaerts, K., Standaert, A. R., and Van Impe, J. F. (2005) Cell division theory and individual-based modeling of microbial lag Part I. The theory of cell division. *Int. J. Food Microbiol*. **101**, 303-318.
8. European Commission. (2000) Report on United States Barriers to trade and Investment. Bruessel, EU.
9. FDA (Food and Drug Administration). (2010) Bacteriological Analytical Manual. Available from: http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm. Accessed Jun. 30. 2010.
10. Gibson, A. M., Bratchell, N., and Roberts, T. A. (1987) The effect of sodium chloride and temperature on the rate and extent of growth of *Clostridium botulinum* type A in pasteurized pork slurry. *J. Appl. Bacteriol*. **62**, 479-490.
11. Gran, H. M., Wetlesen, A., Mutukumira, A. N., Rukure, G., and Narvhus, J. A. (2003) Occurrence of pathogenic bacteria in raw milk, cultured pasteurized milk and naturally soured milk produced at small-scale dairies in Zimbabwe. *Food Control* **14**, 539-544.
12. Halpin-Dohnalek, M. J. and Marth, E. H. (1989) *Staphylococcus aureus*: Production of extracellular compounds and behaviour in foods-a review. *J. Food Protect*. **52**, 67-282.
13. Headrick, M. L., Korangy, S., Bean, N. H., Angulo, F. J., Altekruse, S. F., Potter, M. E., and Klontz, K. C. (1998) The epidemiology of raw milk-associated foodborne disease outbreaks reported in the United States, 1973 through 1992. *Am. J. Public Health*. **88**, 1219-1221.
14. Jay, J. M. (2000) Staphylococcal gatroenteritis. In: Modern food microbiology. Jay, J. M., Loessner, M. J., and Golden, D. A. (ed) Aspen Publishers Inc., Gaithersburg, pp. 441-460.
15. Jung, J. H. and Lee, S. Y. (2010) Microbial growth in dry grain food (sunsik) beverages prepared with water, milk, soymilk, or honey-water. *J. Food Sci*. **75**, M239-M242.
16. Klein, E. (1901) Pathogenic microbes in milk. *J. Hyg*. (Lond). **1**, 78-95.
17. McDonals, K. and Sun, D. W. (1999) Predictive food microbiology for the meat industry: A review. *Int. J. Food Mocrobiol*. **52**, 1-27.
18. McMeekin, T. A., Olley, J. N., Ross, T., and Rathowsky, D. A. (1993) Predictive microbiology: Theory and application. Research Studies Press Ltd., Taunton, Somerset.
19. Medved'ová, A., Valík, L'., Sirotná, Z., and Liptáková, D. (2009) Growth characterization of *Staphylococcus aureus* in milk: a quantitative approach. *Czech J. Food sci*. **27**, 443-453.
20. Nauta, M. J., Litman, S., Barker, G. C., and Carlin, F. (2003) A retail and consumer phase model for exposure assessment of *Bacillus cereus*. *Int. J. Food Microbiol*. **83**, 205-218.
21. Notermans, S. and in't Veld, P. (1994) Microbiological challenge testing for ensuring safety of food products. *Int. J. Food Microbiol*. **24**, 33-39.
22. Rall, V. L. M., Vieira, F. P., Rall, R., Vieitis, R. L., Fernandes Jr., A., Candeias, J. M. G., Cardoso, K. F. G., and Araújo Jr., J. P. (2008) PCR detection of staphylococcal enterotoxin genes in *Staphylococcus aureus* strains isolated from raw and pasteurized milk. *Vet. Microbiol.* **132**, 408-413.
23. Roberts, T. A. (1997) Microbial growth and survival: Developments in predictive modeling. *Food Technol*. **51**, 88-90.
24. Ross, T. **(**1996) Indices for performance evaluation of predictive models in food microbiology. *J. Appl. Bacteriol*. **81**, 501-508.
25. Ross. T., Dalgaard, P., and Tienungoon, S. (2000) Predictive modeling of the growth and survival of *Listeria* in fishery products. *Int. J. Food Microbiol*. **62**, 231-245.
26. Steele, M. L., McNab, W. B., Poppe, C., Griffiths, M. W., Chen, S., Degrandis, S. A., Fruhner, L. C., Larkin, C. A., Lynch, J. A., and Odumeru, J. A. (1997) Survey of Ontario bulk tank raw milk for food-borne pathogens. *J. Food Protect*. **60**, 1341-1346.
27. Tamplin, M. L., Paoli, G., Marmer, B. S., and Phillips, J. (2005) Models of the behavior of *Escherichia coli* O157:H7 in raw sterile ground beef stored at 5 to 46°C. *Int. J. Food Microbiol*. **100**, 335-344.

28. Yoon, Y., Georgnaras, I., Kendall, P. A., and Sofos, J. N. (2009) Modeling the effect of marination and temperature on *Salmonella* inactivation during drying of beef jerky. *J. Food Sci.* **74**, M165-M171.
29. Zhao, L., Chen., Y., and Schaffner, D. W. (2001) Comparison of logistic regression and linear regression in modeling percentage data. *Appl. Environ. Microbiol*. **67**, 2129-2135.
30. Zwietering, M. H., de Wit, J. C., and Notermans, S. (1996) Application of predictive microbiology to estimate the number of Bacillus cereus in pasteurized milk at the point of consumption. *Int. J. Food Microbiol*. **30**, 55-70.

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