

Application of Probabilistic Model to Calculate Probabilities of *Escherichia coli* O157:H7 Growth on Polyethylene Cutting Board

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Abstract

This study calculated kinetic parameters of *Escherichia coli* O157:H7 and developed a probabilistic model to estimate growth probabilities of *E. coli* O157:H7 on polyethylene cutting boards as a function of temperature and time. The surfaces of polyethylene coupons (3×5 cm) were inoculated with *E. coli* O157:H7 NCCP11142 at 4 Log CFU/cm². The coupons were stored at 13 to 35°C for 12 h, and cell counts of *E. coli* O157:H7 were enumerated on McConkey II with sorbitol agar every 2 h. Kinetic parameters (maximum specific growth rate, Log CFU/cm²/h; lag phase duration, h; lower asymptote, Log CFU/cm²; upper asymptote, Log CFU/cm²) were calculated with the modified Gompertz model. Of 56 combinations (temperature×time), the combinations that had ≥0.5 Log CFU/cm² of bacterial growth were designated with the value of 1, and the combinations that had increases of <0.5 Log CFU/cm² were given the value 0. These growth response data were fitted to the logistic regression to develop the model predicting probabilities of *E. coli* O157:H7 growth. Specific growth rate and growth data showed that *E. coli* O157:H7 cells were grown at 28-35°C, but there were no obvious growth of the pathogen below 25°C. Moreover, the developed probabilistic model showed acceptable performance to calculate growth probability of *E. coli* O157:H7. Therefore, the results should be useful in determining upper limits of working temperature and time, inhibiting *E. coli* O157:H7 growth on polyethylene cutting board.

Key words: *Escherichia coli* O157:H7, probabilistic model, predictive model, cutting board

Introduction

Food safety management system such as Hazard Analysis and Critical Control Point (HACCP) has been recommended to improve food safety in meat industry (Gounadaki *et al.*, 2008). For HACCP system, critical control point is a procedure at which controls can be applied, and a food safety hazard can be prevented or reduced to acceptable levels (Bryan, 1990). In meat cutting plant or butcher's shop handling raw meat as a final product, there is no step to reduce the microbiological hazard. Thus, it is important to reduce the bacterial contaminations such as *Salmonella*, *Escherichia coli* O157:H7 and *Campylobacter* spp. through operational hygiene procedures (FSA, 2011). To maintain the good hygiene practices, temperature is one of the important factors for food safety management in butcher's shops in Korea, and the temperature should

be below 15°C in working area of butcher's shops not to allow *Salmonella* and *E. coli* O157:H7 growth (QIA, 2009). For this temperature, a refrigeration system needs to be installed for whole working area, but it is very costly for small-scale butcher's shops. Hence, the data for upper limits of temperature and working hours which can inhibit bacterial growth on food-contact surfaces should be useful information for small-scale butcher's shops. If bacterial growth on food-contact surfaces is inhibited at the certain temperature which can be obtained by air conditioning system, the temperature limit could be reestablished at higher than 15°C with limited working hour for small-scale butcher's shops.

Food-contact surface is a major concern in food processing because they could be a cross-contamination source for foods, and biofilms are also colonized on food-contact surfaces and it exhibits increased antimicrobial resistance (Yoon and Sofos, 2008; Zhao *et al.*, 2011). Tang *et al.* (2011) showed that 44.9% and 49.3% of *Campylobacter jejuni* were transferred from wood and polyethylene cutting boards to chicken fillets.

To describe kinetic behavior of foodborne bacteria on

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food-contact surfaces under different temperatures and time, mathematical modeling is an appropriate tool because mathematical models have been used to estimate kinetic parameters, and the parameters are used to describe kinetic behavior of pathogens (Baranyi and Roberts, 1994; Koutsoumanis *et al.*, 2010). Predictive microbiology has been used to predict inactivation or growth of bacteria, and growth limits of foodborne pathogens in food-like environments and real foods, but not in food-contact surfaces (Deboosere *et al.*, 2010; Mataragas *et al.*, 2006). Hence, the use of predictive models to estimate the food safety related to food-contact surface should be practical application.

Probabilistic models can be used to predict the probability of bacterial growth under different conditions (Tienungoon *et al.*, 2000). To model interfaces between growth and no growth of pathogenic bacteria at certain level of probability, logistic regression analysis can be a useful method (Koutsoumanis *et al.*, 2004; Ratkowsky and Ross, 1995). The concept of probabilistic model was introduced by Genigeorgis *et al.* (1971) and Yoon *et al.* (2011) recently applied the concept to calculate probabilities of *Listeria monocytogenes* growth or no growth on various ready-to-eat meat and poultry products using data from real foods rather than broth media. The calculated probabilities of growth in many combinations of food-related factors could be practical to establish food safety regulations.

Therefore, the objectives of this study were to describe kinetic behavior of *E. coli* O157:H7 and to calculate working temperature and time inhibiting *E. coli* O157:H7 growth on polyethylene cutting board, using a probabilistic model.

Materials and Methods

Preparation of inoculum

E. coli O157:H7 NCCP11142 stored as a frozen culture at -70°C was cultured in 10 mL of tryptic soy broth (TSB; Difco, Becton Dickinson and Company, USA) at 35°C for 24 h. The 0.1 mL of the culture was then transferred into 10 mL of TSB followed by subculture at 35°C for 24 h. Stationary phase cells were harvested by centrifugation (1,912 g, 15 min, 4°C), washed, resuspended in phosphate buffered saline (PBS, pH 7.4; 0.2 g of KH_2PO_4 , 1.5 g of $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, 8.0 g of NaCl, and 0.2 g of KCl in 1 L of distilled water), and diluted in PBS to yield approximately 5 Log CFU/mL of inoculum.

Inoculation

To simulate cutting boards in a butcher's shop, polyethylene coupons (3×5 cm) were swabbed with fresh pork belly pieces using 10 horizontal and 20 vertical passes (1 pass defined as the down and back motion of the hand), and pork purge was sprayed overhead of coupons using a 1 L trigger sprayer; pork purge was prepared by mixture of pork belly and sterile distilled water at ratio of 1 to 1, followed by pummeling (BagMixer[®], Interscience, France). A volume of 0.1 mL of *E. coli* O157:H7 inoculum was introduced over one side of coupons with a sterile bent glass rod, left to stand for 15 min under laminar flow for cell attachment. The target inoculation level was 4-5 Log CFU/cm². The inoculated coupons were placed on plastic containers with lid open and stored at 13, 15, 20, 25, 28, 30, 33 and 35°C for 12 h, and pork purge was sprayed over the coupons after 30 min of inoculation and the same procedure was then applied every hour to simulate smeared purge on cutting boards from repeated fabrication.

Microbiological analysis

Bacterial cell counts of *E. coli* O157:H7 on polyethylene coupons were determined every 2 h for 12 h. To sample surface of coupons, sterile swabs tipped with cotton (Easy swab[®], Komed Co. Ltd., Korea) were used. Briefly, the cap was removed from the vial containing the swab tip and 10 mL of a solution (pH 7.3; 8.0 g sodium chloride, 0.2 g potassium chloride, 0.272 g monopotassium phosphate, 1.42 g disodium phosphate in 1 L of distilled water), and the dampened swab with the solution was used to swab the surfaces with 10 horizontal and 20 vertical passes. Decimal dilutions were made with 9 mL of buffered peptone water (Difco, USA), and 0.1 mL portions of diluents were surface plated on McConkey with sorbitol II agar (Difco, USA). The agar plates were incubated at 35°C for 48 h.

Kinetic parameters

The study was repeated twice (replication) with two samples per replication (n=4). Microbiological data (CFU/cm²) were transformed into Log₁₀ CFU/cm² before being analyzed. Data sets from *E. coli* O157:H7 growth were fitted to the modified Gompertz model (Gibson *et al.*, 1987) to determine the growth kinetic parameters such as maximum specific growth rate (μ_{max} : Log CFU/cm²/h), lag phase duration (LPD: h), lower asymptote (N_0 : Log CFU/cm²), and upper asymptote (N_{max} : Log CFU/cm²). The modified Gompertz model was

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

where N_t is the cell number at any time t , A is the lower asymptotic line of the growth curve as t decreases to zero, C is the difference between the upper asymptotic line of the growth curve and the lower asymptotic line, B is the relative maximum growth rate at time M , and M is the time at which the growth rate is maximum (h). Then, μ_{\max} , LPD, and N_{\max} can be calculated by the equations

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

where e is 2.7182, and

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

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

Evaluation of growth or no growth

A total of 56 treatment combinations of storage temperature (13, 15, 20, 25, 28, 30, 33, and 35°C) and sampling time (0, 2, 4, 6, 8, 10, and 12 h) were studied. The combinations that allowed increases in cell counts of *E. coli* O157:H7 of at least 0.5 Log CFU/cm² compared to the cell counts of *E. coli* O157:H7 on day 0 were assigned the value of 1 (growth), while the combinations that had increases in cell counts of *E. coli* O157:H7 of less than 0.5 Log CFU/cm² compared to *E. coli* O157:H7 cell counts on day 0 were given the value of 0 (no growth). Threshold levels of the pathogen cell counts to determine growth and no growth could be altered according to a company's product standard or other considerations, including regulatory standards (Yoon *et al.*, 2011).

Probabilistic model development

The growth response data (values of 1 or 0) were used to develop a probabilistic model to predict *E. coli* O157:H7 growth probabilities, using the logistic regression analysis of SAS[®] version 9.2 (SAS Institute Inc., USA) (Ratkowsky and Ross, 1995). Following equation was derived using the logistic regression analysis, and significant parameters were selected with a stepwise selection method ($p < 0.05$) (Koutsoumanis *et al.*, 2004; McKellar and Lu, 2001).

$$\text{Logit}(P) = a_0 + a_1 \cdot \text{Temp} + a_2 \cdot \text{Time} + a_3 \cdot \text{Temp}^2 + a_4 \cdot \text{Time}^2 \quad (5)$$

where $\text{Logit}(P)$ is an abbreviation of $\ln[P/(1-P)]$, P is the probability of growth (in the range of 0 to 1), a_i are estimates, Temp is storage temperature, and Time is storage time.

Model validation

The growth probabilities of *E. coli* O157:H7 predicted by the model were compared to the observed bacterial populations from other study conducted in our laboratory. In the available data, if a more than 0.5 Log unit increase was observed during storage, it was defined as 'growth' and if a less than 0.5 Log increase in growth or a decline was observed, it was defined as 'no growth'.

Results and Discussion

Kinetic behavior of *E. coli* O157:H7

Initial bacterial populations of *E. coli* O157:H7 on polyethylene coupons were approximately 4.0 Log CFU/cm² (Table 1; Fig. 1). Obvious growth of *E. coli* O157:H7 on the coupons were not observed during storage at 13, 15, 20, and even at 25°C for 12 h (data not shown), but gradual increases in bacterial cell counts of the pathogen were observed approximately up to 5.7 to 7.1 Log CFU/cm² at 28, 30, 33, and 35°C (Table 1 and Fig. 1). Hence, observed growth data only from 28, 30, 33, and 35°C were fitted to the modified Gompertz model to calculate kinetic parameters, and as expected, *E. coli* O157:H7 incubated at 28°C had longer LPD, and lower μ_{\max} and N_{\max} values than 30-35°C (Table 1 and Fig. 1). The low N_{\max} value at 28°C may be caused by decreased affinity for substrates at low temperature because low temperature may inhibit active transport of bacterial cells (George *et al.*, 1996; McClure *et al.*, 1997).

Coefficient of determination (R^2) ranged from 0.821 to 0.969 after fitting the observed data to the modified Gompertz model, and all predicted growth curves passed through most observed data points, regardless of storage temperature (Table 1 and Fig. 1). This indicates that the kinetic model adequately describes biological behavior of *E. coli* O157:H7 on polyethylene coupons, although the R^2 values are varied. This variation of R^2 could be caused

Table 1. Kinetic parameters (mean \pm standard deviation) calculated by the modified Gompertz model for *Escherichia coli* O157:H7 on polyethylene coupons

Temperature (°C)	LPD (d)	μ_{\max} (Log CFU/cm ² /h)	N_0 (Log CFU/cm ²)	N_{\max} (Log CFU/cm ²)	R^2
28	7.20 \pm 0.0	0.42 \pm 0.0	4.2 \pm 0.2	5.7 \pm 0.0	0.821
30	4.31 \pm 1.08	0.52 \pm 0.26	4.3 \pm 0.0	7.1 \pm 0.1	0.969
33	4.94 \pm 0.42	2.06 \pm 1.08	3.8 \pm 0.1	6.5 \pm 0.1	0.922
35	5.89 \pm 2.07	1.32 \pm 1.3	3.9 \pm 0.3	6.6 \pm 0.7	0.840

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

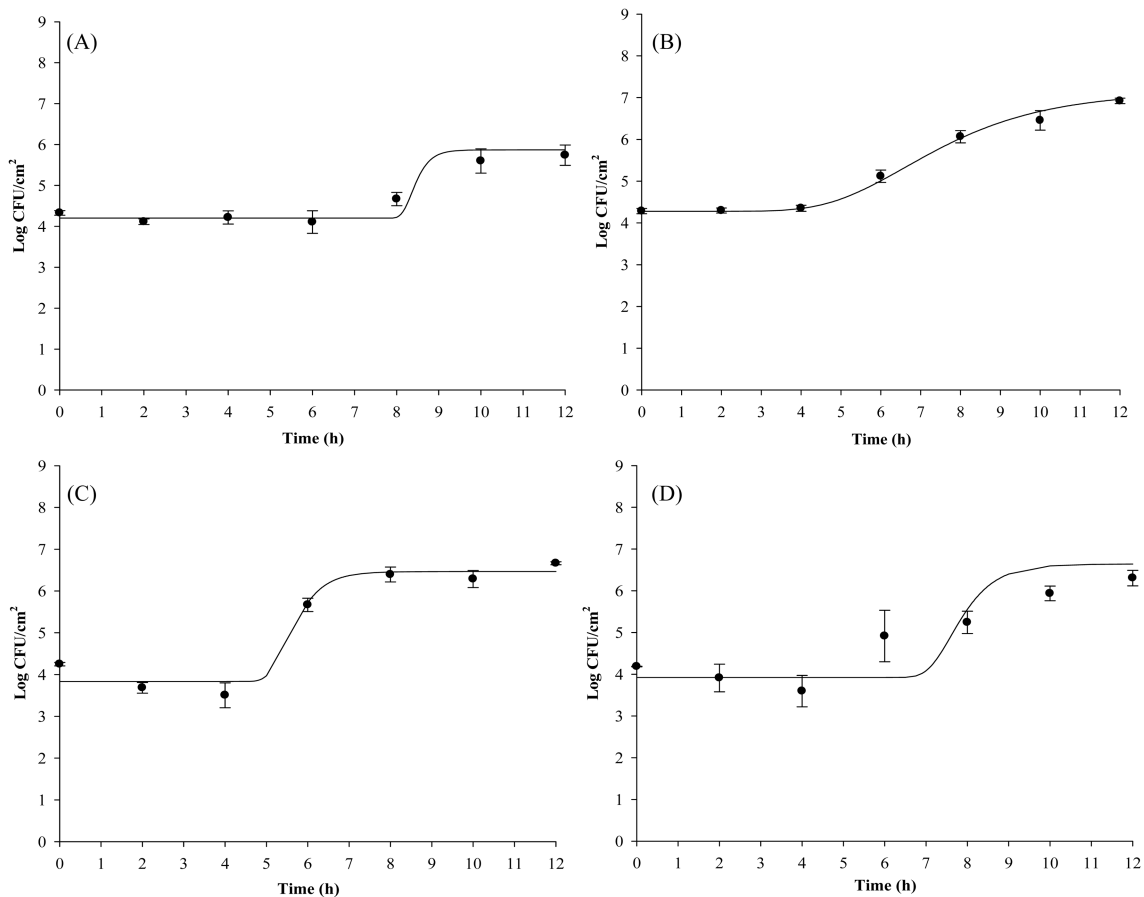


Fig. 1. Bacterial populations (symbols) of *Escherichia coli* O157:H7 recovered with McConkey with sorbitol II agar from polyethylene coupons and predicted cell counts (line) by the modified Gompertz model during 12 h of incubation at 28°C (A), 30°C (B), 33°C (C), and 35°C (D); only temperatures, which showed growth of the pathogen, are presented.

by the competition between various natural flora and *E. coli* O157:H7 called the James effect, which is a race between bacterial species to use the resources of the environment (Coleman *et al.*, 2003; Cornu *et al.*, 2011). The majority of mathematical equations have been used for fitting data from liquid laboratory media which produce good fitting results, but interactions between indigenous microflora and pathogens in foods and food-related conditions should be considered to develop practical models because indigenous microflora may influence fates of foodborne pathogens (Cornu *et al.*, 2011; Yoon *et al.*, 2009).

According to the results from Fig. 1, following implication can be introduced. Even though *E. coli* O157:H7 cells are contaminated on polyethylene cutting board, the pathogen may not proliferate up to 12 h, if cells of the pathogen are exposed to less than 25°C.

Probabilistic model

To calculate growth probability of *E. coli* O157:H7 on polyethylene coupons, estimates of coefficients were

determined by stepwise selection method after analyzing binary growth response data with the logistic regression analysis (Table 2). The estimates were then used to calculate probabilities of *E. coli* O157:H7 growth on polyethylene coupons, and the predicted interfaces between growth and no growth of *E. coli* O157:H7 at 0.1, 0.5, and 0.9 of probabilities were produced as a function of temperature and time (Fig. 2). This result can be used to predict combinations of upper limits of working temperature and exposure time to inhibit *E. coli* O157:H7 growth on polyethylene cutting board at a desired probability of

Table 2. Estimates of parameters selected from the logistic regression analysis by a stepwise selection method to calculate growth probabilities of *Escherichia coli* O157:H7 on polyethylene coupons

Coefficient	Estimate	Standard error	<i>p</i> -value
Intercept	-242.1	63.0621	0.0001
Temperature	14.6311	3.9472	0.0002
Time	1.5252	0.3600	<0.0001
Temperature×Temperature	-0.2284	0.0632	0.0003

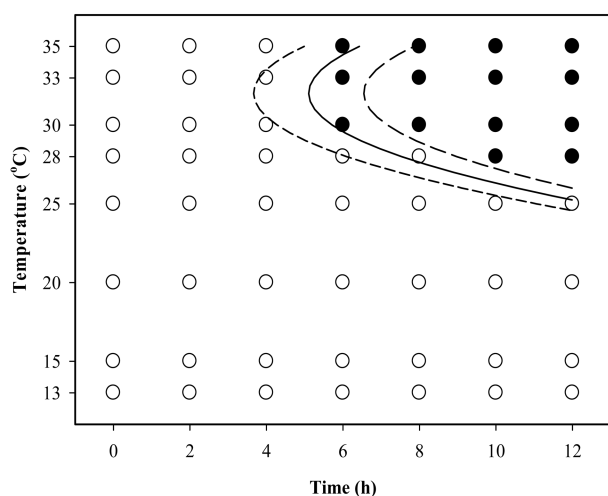


Fig. 2. The observed growth response (growth: ●, no growth: ○) of *Escherichia coli* O157:H7 on polyethylene coupons stored at 13, 15, 20, 25, 28, 30, 33, and 35°C up to 12 h, and interfaces between growth and no growth with respect to temperature and time as predicted by the developed probabilistic model using the equation 5 at growth probabilities of 0.1 (lower line), 0.5 (middle line), and 0.9 (upper line).

growth. For instance, *E. coli* O157:H7 started to grow after 10 h of incubation at between 25°C and 28°C at 0.5 of probability, and at higher incubation temperature *E. coli* O157:H7 growth was observed between 6 and 8 h of incubation at 0.5 probability (Fig. 2). If the probability of 0.5 is considered liberal, more conservative probability can be used (Tienungoon *et al.*, 2000). This indicates that with consideration of the growth inhibiting *E. coli* O157:H7 on cutting boards the temperature in working area of butcher's shops should be determined according to the length of working hour rather than applying only 15°C as indicated in the regulation of QIA (2009).

Probabilistic model performance

Goodness of fit of the developed probabilistic model was measured by the concordance index showing the degree of agreement between the predicted probabilities and the observed responses (Koutsoumanis *et al.*, 2004). For the developed probabilistic model in this study, the degree of agreement between the predicted probabilities and the observations was 99.3% and 0.6% of discordance was observed (data not shown). For further evaluation of model performance, the predicted growth probabilities calculated by the developed model were compared to the observed growth response data on which the model was based (Koutsoumanis *et al.*, 2004). This result showed that one observed growth response (1.79 %) of 56 combinations disagreed with the model prediction; in the model

Table 3. Comparison between observed responses of *Escherichia coli* O157:H7 on polyethylene coupons and growth probability of the pathogen predicted by the developed probabilistic model

Temperature (°C)	Time (h)	Observed response	Predicted probability of growth
18	9	No growth	0.0
	1	No growth	0.0
28	5	No growth	0.0
	9	Growth	0.903
	11	Growth	0.995
32	3	No growth	0.0
	7	Growth	0.947
	9	Growth	0.997
	11	Growth	1.0
35	1	No growth	0.0
	5	No growth	0.102
	7	Growth	0.706
	11	Growth	0.999

prediction growth or no growth of the combinations were determined at 0.5 of growth probability (Koutsoumanis *et al.*, 2004; Yoon *et al.*, 2009; Yoon *et al.*, 2011). The developed probabilistic model predicting growth probability of *E. coli* O157:H7 was validated with the data from other study in our laboratory, and the model displayed agreement with most growth responses of *E. coli* O157:H7 on polyethylene coupons (Table 3).

In conclusion, this study provides quantitative data of *E. coli* O157:H7 growth on the polyethylene cutting board incorporating working hour and temperature, and the interfaces between growth and no growth of the pathogen at various probabilities. Therefore, the results should be useful in determining upper limits of working temperature and time not to allow *E. coli* O157:H7 growth on polyethylene cutting board, which could be useful information in food safety management system, especially for small-scale butcher's shops.

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