# DEVELOPMENT OF A DYNAMIC MODEL TO PREDICT THE FATE OF PATHOGENIC *ESCHERICHIA COLI* IN DICED CUCUMBER UNDER CHANGING TEMPERATURES

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## ABSTRACT

*Escherichia coli* has been detected in a variety of foods, particularly in salad vegetables, such as diced cucumbers. However, it is difficult to control this pathogen in salad vegetables, because they are consumed without additional preparation or cooking. Thus, the objective of this study was to develop dynamic models to describe the kinetic behavior of *E. coli* in diced cucumber. The diced cucumber was inoculated with *E. coli*, and stored at 10°C, 20°C, 25°C, and 30°C; cells counts were then performed using Petrifilm™ plates. The Baranyi model was used to calculate lag phase duration (*LPD*; h) and maximum specific growth rate ($\mu_{max}$.; log CFU/g/h). These parameters were then fitted to a polynomial model, as a function of temperature, and a subsequent dynamic model was developed in accordance with these primary and secondary models. The performance of the model was evaluated by comparing predicted data with observed data to calculate the root mean square error (*RMSE*). As temperature increased, *LPD* decreased, but $\mu_{max}$ increased. The secondary model effectively described the temperature effect on *LPD* and $\mu_{max}$, where $R^2$ equaled 0.972-0.983. In the validation stage, *RMSE* value of 0.272 suggested that model performance was appropriate to predict cell counts in diced cucumber, and these predictions remained appropriate under changing temperatures. These results indicate that *E. coli* can grow rapidly in diced cucumber at high storage temperatures, and present a useful dynamic model for describing the kinetic behavior of *E. coli* in this vegetable.

*Keywords*: *Escherichia coli,* cucumber, mathematical model, dynamic model

## 1. INTRODUCTION

Interest in health and diet has led to an increase in the production and consumption of fresh vegetables in recent years (WIRSENIUS *et al*., 2010; VEREECKEN *et al*., 2015). A survey conducted by NGUYEN *et al*. (2015) reported that 26% of foodborne illness is due to consumption of contaminated fruits and vegetables. Although consumers usually think that fruit and vegetable salads are microbiologically safe, foodborne pathogens can survive and replicate in fresh vegetables (CALLEJÓN *et al*., 2015; BENNETT *et al*., 2018). In addition, fresh vegetables are eaten raw and can, therefore, be more dangerous than other food products, following exposure to foodborne bacteria. Specifically, there have been many cases of food poisoning due to contamination of sliced cucumber (DECRAENE *et al*., 2012; ANGELO *et al*., 2015).

*Escherichia coli* is a facultative anaerobic, Gram-negative bacillus that belongs to the Enterobacteriaceae family (PATERSON, 2006; STEPIEN-PYSNIAK, 2010). *E. coli* is considered an indicator organism for contamination (CHOI *et al*., 2018) and major cause of foodborne illness, particularly via contamination of fresh vegetables by enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), and enteroaggregative *E. coli* (EAEC) (NATARO and KAPER 1998; OLSEN *et al*., 2000; JANG *et al*., 2017). Among these pathotypes, ETEC is the most frequent cause of food poisoning and outbreaks caused by EHEC have been emerging in recent years (GOULD *et al*., 2013; CATFORD *et al*., 2014).

Predictive microbiology is a strategy that employs mathematical models to estimate the kinetic parameters of foodborne pathogens, and is aimed at securing food safety via the prevention of potential risks or hazards (WHITING and BUCHANAN, 1997; YOON, 2010). Most predictive models are developed using a constant temperature (HA *et al*., 2019; LEE *et al*., 2019); however, a number of variables, such as temperature and humidity, change during food storage and distribution. For this reason, a dynamic model should be used to describe the fate of foodborne pathogens under these changing conditions (HA *et al*., 2015; CHOI *et al*., 2016).

Therefore, the objective of this study was to develop a dynamic model to describe the kinetic behavior of *E. coli* in diced cucumber at a range of temperatures.

## 2. MATERIALS AND METHODS

### 2.1. *E. coli* prevalence in cucumbers

To evaluate *E. coli* contamination levels, 24 cucumbers were purchased from conventional markets or grocery stores in Korea. Twenty five-gram portions of cucumber were placed into sterile filter bags (3M, St. Paul, MN, USA), and 225 mL 0.1% buffered peptone water (BPW; Becton Dickinson and Company, BD, Franklin Lakes, NJ, USA) was added prior to homogenization for 60 sec. For quantitative analysis of *E. coli*, 1 mL of the homogenate was dispensed into a Petrifilm™ *E. coli*/Coliform Count Plate (3M, USA), which was then incubated at 37°C for 24 h. Any blue colonies with associated gas bubbles were identified and counted. For qualitative analysis of *E. coli*, 1 mL of the homogenate was added to *E. coli* (EC) broth (BD, USA) containing a durham tube and cultured at 44.5°C for 24-48 h. The aliquot in the gas-producing tube was then streaked on eosin methylene blue (EMB) agar (BD, USA) and incubated at 37°C for 24-48 h. The typical colonies representing *E. coli*, with a green color and metallic sheen, were counted. Finally, the 16S rRNA gene was

amplified using the following primers: 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1492R (5'-TAC GGY TAC CTT GTT ACG ACT T-3') and sequenced for identification. To determine if the *E. coli* isolates were pathogenic, genomic DNA was extracted using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) and amplified by PCR, using a Powerchek™ Diarrheal *E. coli* 8-plex Detection Kit (Kogene Biotech, Seoul, Korea), according to the manufacturers' protocols. The following PCR conditions were used: initial denaturation at 95°C for 12 min, followed by 32 cycles of 95°C for 30 sec, 60°C for 45 sec, 72°C for 60 sec, and a final extension at 72°C for 10 min. The characteristics of the genes used in PCR are shown in Table 1. To confirm amplification of the target gene, the PCR product was resolved on a 1.5% agarose gel in 1X TAE buffer (Biosesang, Seongnam-si, Korea).

### 2.2. Preparation of inocula

*E. coli* (NCCP14038, NCCP14039, NCCP15661, and NCCP11142) were cultured in 10 mL tryptic soy broth (TSB; BD, USA) at 37°C for 24 h. One milliliter of culture was transferred into 10 mL fresh TSB and subcultures were incubated at 37°C for 24 h. The subcultured strains were mixed into a tube, and then centrifuged at 1,912 ×*g* at 4°C for 15 min. The supernatants were discarded, and the cell pellets were washed twice with phosphate-buffered saline (PBS: pH 7.4; 0.2 g $KH_2PO_4$, 1.5 g $Na_2HPO_4{\cdot}7H_2O$, 8.0 g NaCl, and 0.2 g KCl in 1 L distilled water). The cell pellets were then resuspended in PBS, and further diluted with PBS to a final concentration of 5 log CFU/mL for inoculation.

### 2.3. Development of predictive models

Cucumbers were diced into 25-g portions, then dipped into the pathogenic *E. coli* inocula for 3 min, drained for 10 min, and then placed in a sterile bag. The samples were stored at 10°C, 20°C, 25°C, and 30°C up to 96 h, depending on storage temperature. To enumerate *E. coli* in the cucumbers, the samples were aseptically transferred to sample bags (3M, USA) containing 225 mL BPW and homogenized using a pummeler (BagMixer; Interscience, St. Nom, France). The homogenates were serially diluted in BPW and 1 mL of each dilution was transferred to a Petrifilm™ Plate. The plates were incubated at 37°C for 24 h and the colonies were then manually counted. The experiment was repeated three times for each temperature. The primary model was developed by fitting the cell count data to the Baranyi model, using DMFit curve fitting software (Institute of Food Research, Norwich, UK) to calculate lag phase duration (*LPD*; h) and maximum specific growth rate ($\mu_{max}$; log CFU/g/h). The equation was as follows:

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

where $N_t$ is the bacterial cell count at time *t*, and $N_0$ and $N_{max}$ are the initial and final bacterial cell counts in a growth curve, respectively. $A_t$ is the adjustment function, which denotes the physiological status of bacterial cells when defining the *LPD* (BARANYI and ROBERTS 1994). *LPD* and $\mu_{max}$ values were further-analyzed using a polynomial model as a function of temperature to develop a secondary model as follows:

$$LPD \text{ or } \mu_{max} = a_0 + a_1 T + a_2 T^2 \quad \text{Eq. 2}$$

where $a_i$ are the coefficient values and $T$ is the storage temperature (°C).
Also h0 values were calculated for describing the initial physiological status of bacterial cells.

## 2.4. Validation

To evaluate model performance, additional experiments were performed at 15°C and 23°C. During storage, "observed data" for *E. coli* cell counts were obtained as described above. These observed data were then compared to the predicted data, calculated using the model.

**Table 1.** Target genes for *Escherichia coli* pathogen type with PCR.

| Pathogen | Target gene | Size (bp) |
|---|---|---|
| EAEC[1)] | *aggR* | 757 |
| EHEC[2)] | *VT1 (stx1)* | 637 |
| ETEC[3)] | *LT* | 530 |
| EPEC[4)] | *bfpA* | 400 |
| EHEC | *VT2 (stx2)* | 297 |
| EPEC | *eaeA* | 231 |
| ETEC | *ST (STh/STp)* | 167 |
| EIEC[5)] | *ipaH* | 141 |

[1)]Enteroaggregative *E. coli.* [2)] Enterohemorrhagic *E. coli.* [3)]Enterotoxigenic *E. coli.* [4)]Enteropathogenic *E. coli.* [5)]Enteroinvasive *E. coli.*

The differences between the observed and predicted data were quantified by calculating the root mean square error (*RMSE*), bias factor (*B* factor) and accuracy factor (*A* factor) as follows:

$$RMSE = \sqrt{1/\mathrm{n} \times \sum(\text{observed data} - \text{predicticed data})^2} \quad \text{Eq. 3}$$

$$B \text{ factor} = 10^{[\sum \log((predictive\ values/observed\ values)/n]} \quad \text{Eq. 4}$$

$$A \text{ factor} = 10^{[\sum |\log(predictive\ values/observed\ values)|/n]} \quad \text{Eq. 5}$$

where $n$ represents the number of data points.

## 2.5. Development of a dynamic model

To describe the *E. coli* growth in cucumbers at changing temperatures, a dynamic model was developed with the equation suggested by BARANYI and ROBERTS (1994), in accordance with primary and secondary models detailed above. To evaluate the performance of the dynamic model, *E. coli*-inoculated cucumber samples were stored at fluctuating temperatures (10°C-28°C), and cell counts were performed as described above.

These cell counts were then compared with the predicted cell counts generated using the dynamic model.

### 2.6. Statistical analysis

*LPD* and $\mu_{max}$ data were analyzed with a general linear model using SAS® software version 9.4 (SAS Institute, Inc., Cary, NC, USA). The mean comparisons among storage temperature were performed using a pairwise *t*-test at $\alpha = 0.05$.

## 3. RESULTS AND CONCLUSIONS

*E. coli* and coliform bacteria were not detected in the quantitative analysis of cucumber samples, although *E. coli* was detected in one sample by qualitative analysis. This organism was identified as non-pathogenic *E. coli* via 16S rRNA gene sequencing (Fig. 1). *E. coli* has been detected in salad vegetables in previous studies (VISWANATHAN and KAUR, 2001; RAHMAN and NOOR, 2012), and specifically, food poisoning associated with cucumbers has been reported in Canada and Sweden (DECRAENE *et al*., 2012; KOZAK *et al*., 2013). Thus, *E. coli* can be considered as an important risk in cucumbers and the behavior of this bacteria in cucumbers should be investigated.

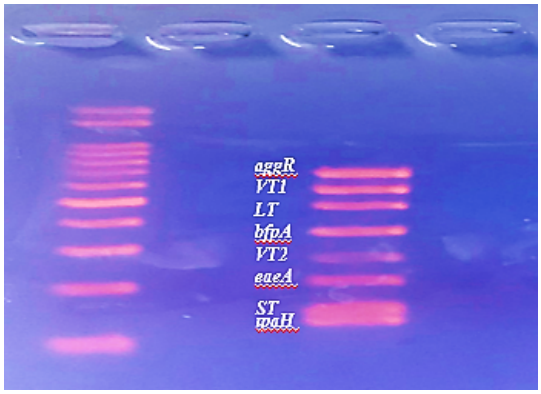


**Figure 1.** Multiplex PCR results for *Escherichia coli* pathogen type isolated from cucumbers using primers targeting *aggR, VT1, LT, bfpA, VT2, eaeA, ST*, and *ipaH* genes. Lane 1: 100 bp-marker; Lane 2: negative control; Lane 3: positive control; Lane 4: *E. coli* isolated from cucumbers.

Cell counts increased gradually when cucumber samples were stored at 10°C; however the counts increased rapidly in cucumbers stored at 20°C-30°C, reaching stationary phase within 12-24 h, depending on storage temperature (Fig. 2). In addition, *LPD* (1.73-5.00 h) was very short at this temperature range (1.73-5.00 h) (Table 2). Similarly, $\mu_{max}$ was measured as 0.01 log CFU/g/h in samples stored at 10°C, and increased values (0.29-0.42 log CFU/g/h) were recorded from samples stored at 20°C-30°C (Table 2). These results indicate that if cucumbers are contaminated with *E. coli*, the bacteria can replicate and cross contamination can occur during cutting. *E. coli* can grow very quickly in diced cucumber during preparation, indicated by low *LPD* values of 3.10 and 1.73 h for storage at 25°C and 30°C, respectively.

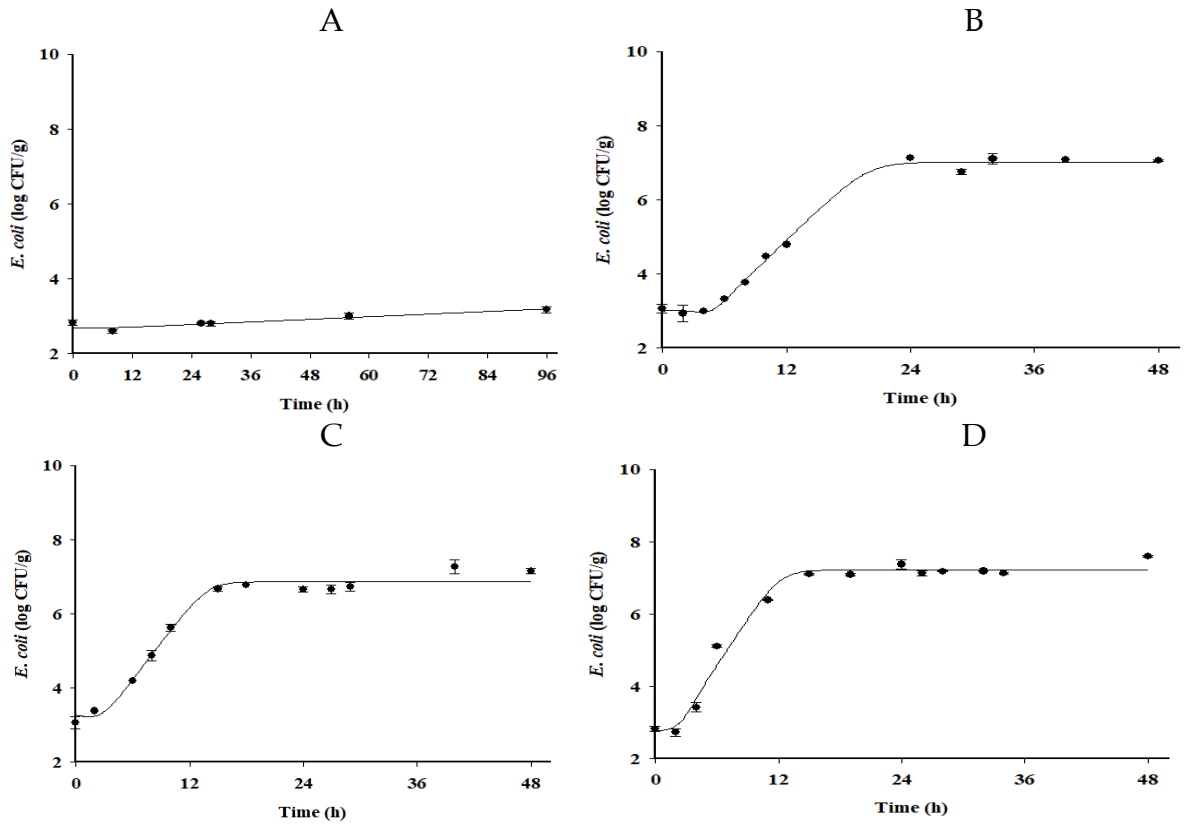


**Figure 2**. Bacterial populations of pathogenic *Escherichia coli* in cucumbers during storage at 10°C (A), 20°C (B), 25°C (C), and 30°C (D) for 96, 48, 48, and 48 h respectively; • observed value, - fitted line.

**Table 2.** The parameters calculated by the Baranyi model for pathogenic *Escherichia coli* growth in cucumber.

| Storage temperature (°C) | *LPD*[1)] (h) | $\mu_{max}$[2)] (log CFU/g/h) | h0[3)] | $N_0$[4)] (log CFU/g) | $N_{max}$[5)] (log CFU/g) | $R^2$ |
|---|---|---|---|---|---|---|
| 10 | 11.15±1.35$^{A}$ | 0.01±0.00$^{D}$ | 0.10 | 2.85±0.16 | 3.47±0.24 | 0.648 |
| 20 | 5.00±0.64$^{B}$ | 0.29±0.03$^{C}$ | 1.45 | 2.98±0.17 | 6.96±0.21 | 0.989 |
| 25 | 3.10±0.03$^{C}$ | 0.37±0.03$^{B}$ | 1.15 | 3.06±0.20 | 7.44±0.49 | 0.983 |
| 30 | 1.73±0.12$^{C}$ | 0.42±0.02$^{A}$ | 0.73 | 3.01±0.20 | 7.48±0.52 | 0.975 |

[1)]Lag phase duration. [2)]Maximum specific growth rate. [3)]Parameter specifying the initial physiological state of cells. [4)]Initial cell concentration. [5)]Maximum cell concentration.
[A-D]Means within the same column with different superscript letters are significantly different ($p$<0.05).

These results are consistent with those observed in a study by ABDUL-RAOUF *et al*. (1993). The h0 (which is a value multiplied by *LPD* and $\mu_{max}$) is the value obtained by quantifying the initial physiological status (BARANYI and ROBERTS, 1994; GRIJSPEERDT and VANROLLEGHEM, 1999; MCKELLAR, 2001); this measure was higher at temperatures above 20°C (0.73-1.45) (Table 2). This indicates that cells grown at temperatures over 20°C can adapt to the actual environment more quickly, and therefore, storing contaminated cucumbers above 20°C may increase the risk.

To evaluate the effect of the temperature on the kinetic parameters (*LPD* and $\mu_{max}$), a secondary model was developed in which $R^2$ was calculated to be 0.972-0.983 (Fig. 3), indicating that this model was appropriate to describe the effect of temperature on kinetic parameters. The *RMSE* value was calculated to evaluate model performance, where a value close to zero indicates that the predicted values are the same as the observed values (KIM *et al.*, 2018). In this study, *RMSE* was calculated as 0.272, indicating that the developed models were appropriate for describing the kinetic behavior of *E. coli* in cucumbers. Also, *B* factor and *A* factor were 0.98 and 1.04, respectively. ROSE (1999) showed that the developed model was suitable if the *B* factor was 0.9 to 1.05 and the *A* factor was below 1.15. Thus, the developed models in our study were appropriate.
Using the model, *E. coli* cell counts were predicted at changing temperatures (10°C-28°C), simulating storage, distribution, and preparation, and these predicted values were similar to the observed cell counts (Fig. 4).

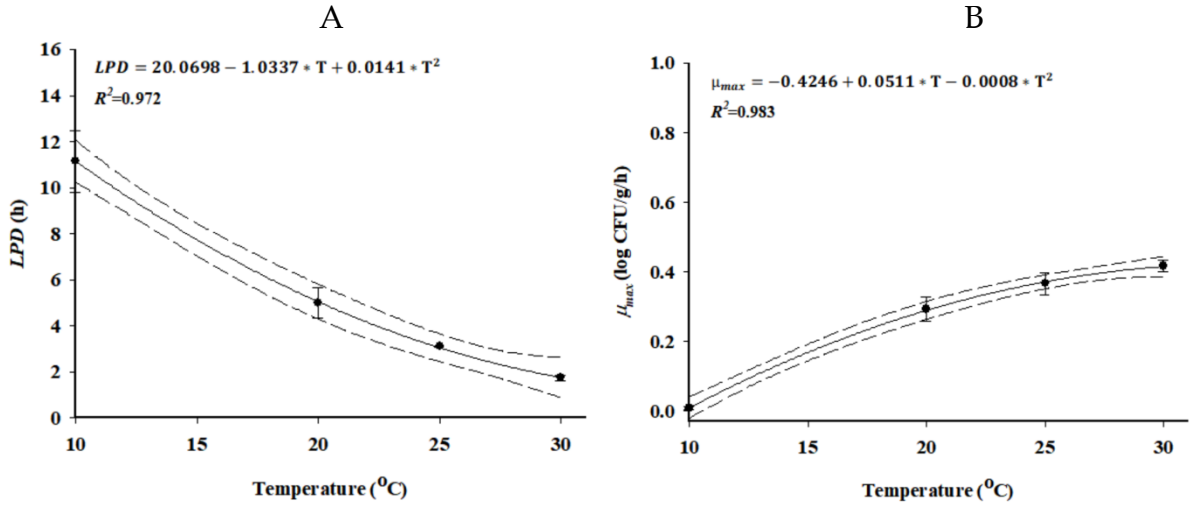


**Figure 3**. Secondary predictive model for lag phase duration (*LPD*) (A) and maximum specific growth rate ($\mu_{max}$) (B) of *Escherichia coli* in cucumbers, as a function of temperature.

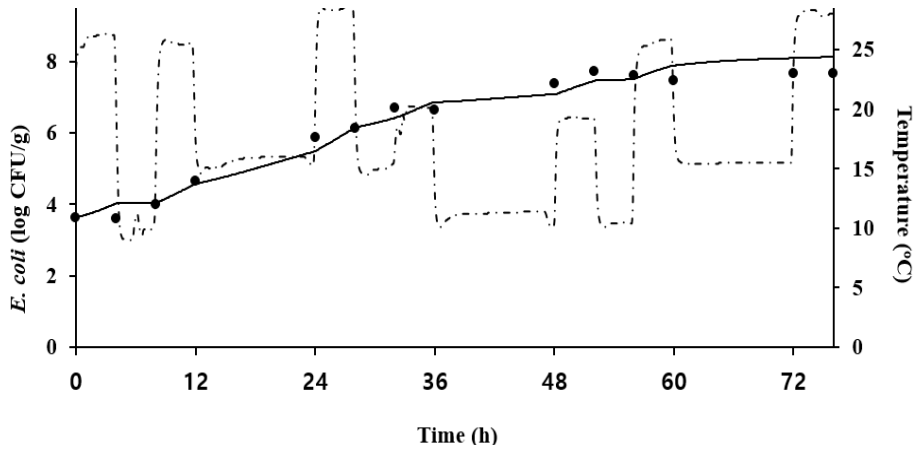


**Figure 4**. Dynamic model for *Escherichia coli* in cucumbers (Symbol: observed cell counts, line: predicted cell counts).

This result indicates that this dynamic model is appropriate for the description of *E. coli* growth in cucumbers even at changing temperature.
In conclusion, *E. coli* can grow rapidly in cucumbers if they exposed to temperatures above 10°C, which can occur during preparation and short-term storage at room temperature. Furthermore, the model developed here should be appropriate to predict *E. coli* growth in cucumbers, particularly under changing temperatures.

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