Predictive model for the growth kinetics of *Listeria monocytogenes* in raw pork meat as a function of temperature

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**Abstract**

This study was performed to develop a predictive growth model of *Listeria monocytogenes* to ensure the safety of raw pork. The pork samples were inoculated with a cocktail of two *L. monocytogenes* strains ATCC 15313 and L13-2 isolated from pork and were stored at 5, 15, and 25 °C. Results were evaluated using the MicroFit program. To develop primary models, the Baranyi, modified Gompertz, and Logistic model equations were applied to the observed data. The mathematically predicted growth rate parameters were evaluated using the coefficient of determination (R²), bias factor (Bf), accuracy factor (Af), and mean square error (MSE). The Baranyi model, which showed an R² of 0.998 and MSE of 0.006, was more suitable than the modified Gompertz and Logistic models. In validation study of secondary model, it appeared that MSE’s of specific growth rate (SGR) and lag time (LT) were relatively accurate and suitable for modeling the growth of *L. monocytogenes*. These values indicated that the developed models were acceptable for expressing the growth of microorganisms on raw pork, which can be applied to ensure the safety of meats and to establish standards for avoiding microbial contamination.

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1. Introduction

Recently, there has been an increase in the frequency of breakouts of foodborne illnesses because of the increased consumption of cold-stored food, particularly meat products (DeWaal, Tian, & Plunkett, 2012). In particular, various meat products have played important roles in the outbreaks frequency of listeriosis in the United Kingdom, United States, and other countries (McLaughlin, 1996). Pork makes up 40% of the meat consumed in the world, followed by poultry and beef. Although the consumption of pork meat products is increasing, the safety of pork during storage or distribution is becoming a serious problem because, while meat products generally have high nutritional value, food poisoning can occur because of careless treatment during processing, storage, and distribution (Aymérich, Picouet, & Monfort, 2008).

*Listeria monocytogenes* is a gram-positive, non-sporing bacillus, which is positive for catalase and Voges–Proskauer and negative for indole and nitrate reduction. Although the optimum temperature for growth is 37 °C, it can grow even at refrigeration temperatures (4–15 °C). Therefore, this microorganism can still cause food poisoning in developed countries that use advanced refrigeration technology for food distribution (Cho, Lee, Lim, Kwak, & Hwang, 2011).

*L. monocytogenes* is a significant foodborne pathogen concern because of the high mortality rate due to listeriosis in people consuming refrigerated meat products (Bennion, Sorvillo, Wise, Krishna, & Mascola, 2008). Furthermore, *L. monocytogenes* has the ability to survive and adapt to adverse environments (Bennion et al., 2008; Gandhi & Chikindas, 2007), and the meat can be re-contaminated during packaging (Sofos, 2008). In the European Union (EU), cases of listeriosis have increased by 19.1% in 2009 compared to 2008, and 270 people died from listeriosis in 2007 (EFSA, 2011, 2012). In the United States, there have been 2500 cases of listeriosis, of which 2289 cases required hospitalization. In 2009, the mortality rate of listeriosis was reported to be 28% (Wesley, Heredia, & Garcia, 2009, pp. 3–13). For healthy adults,
the dose of \( L. \) monocytogenes required to cause listeriosis has been reported to vary from \( 10^3 \) to \( 10^9 \) CFU/g (Dalton et al., 1997; Miettinen et al., 1997). However, for high-risk groups, infectious doses between \( <10 \) and \( 10^4 \) CFU/g have been reported (Berrang, Frank, & Brackett, 1988; Ericsson et al., 1997). In particular, the acceptable level of \( L. \) monocytogenes is regulated in foods to \( <10^2 \) CFU/g at the time of consumption in Europe (Anonymous, 2005).

Recently, predictive estimation methods for foodborne pathogens in food products have been studied using a mathematical description of the growth and survival of microorganisms with respect to food safety (Hong et al., 2005). Predictive models can be used to estimate variations in the pathogen density at any given time and temperature in the processing operations during storage and distribution. Traditional microbiological techniques are time-consuming and require a pre-enrichment step to pretreat samples. Although fast, the cost of some rapid enumeration techniques is prohibitively high. Predictive models, on the other hand, can provide an evaluation of the growth of foodborne pathogens in food (Padmanabha et al., 2011). Such predictive models can be useful in the decision making of Critical Control Points (CCP) in HACCP and risk assessment by estimating changes in microbial numbers in a production chain (Fakruddin, Mazumder, & Mannan, 2011). However, the models have been differently applied according to food products and strains (Cho et al., 2011; Lobacz, Kowalik, & Tarczynska, 2013; Moller et al., 2013). In addition, it has been reported that the growth patterns of \( Listeria \) in food products also is different according to strains (Vermeulen et al., 2007).

Therefore, the purpose of this study was to compare three models to develop predictive models for \( L. \) monocytogenes isolated from commercial raw pork in Korea with the variables of temperature and time on raw pork, and to identify the optimal model by evaluating and comparing several models during storage.

2. Materials and methods

2.1. Microbial counting and determination of pH and water activity of pork samples

Pork meat was purchased from major retail market in Seoul. The samples were cut into 10 g portions and aseptically transferred into a sterile stomacher bag and added 90 mL of 0.1% peptone water for examining initial total plate counts. Total plate counts were determined on plate count agar (PCA, Difco Laboratories, Detroit, MI, USA).

The pH measurements were conducted with a pH meter (inolab, Germany) using 10 g of sample mixed with 90 mL of distilled water. The water activity (Aw) was measured by Aquasaptor (NAGY-Instruments.de, Gaeufelden, Germany). Three replicates of each sample were tested.

2.2. Bacterial strains

Two strains of \( L. \) monocytogenes, ATCC 15313 in laboratory and L13-2 isolated from Korean processed pork product (bulgogi) (Ahn, Lee, Lee, & Paik, 2012), were used in this study. These strains were maintained in tryptic soy broth (TSB, Difco Laboratories) containing 20% glycerol at \(-80\) °C. The stock cultures were thawed at room temperature, and then 100 \( \mu \)L was inoculated into 10 mL of TSB and incubated at 35 °C for 24 h for activation. Cultures were grown until the late exponential phase of growth (\( >8 \) log CFU/mL) and serially diluted into 0.1% sterile peptone water for use.

2.3. Inoculation and enumeration

The pork samples were purchased from major retail outlets located in the city of Seoul, Korea, and transported to the lab within 30 m. Purchased pork meat was cut into 10 g portions. The mix of \( L. \) monocytogenes strains (100 \( \mu \)L) was inoculated onto each pork surface. And inoculated pork meats were into the sterile filter bag (Inter Science). The initial cell counts were adjusted to 3 log CFU/g. The inoculated samples were stored at 5, 15, and 25 °C refrigerator to develop the primary growth models. After stored, pork samples were added with 90 mL of 0.1% peptone solution and were homogenized with a homogenizer (IUL instruments, Masticator classic, Barcelona, Spain). These samples were diluted with 90 mL of 0.1% peptone water and plated onto Oxford agar with modified oxoid antimicrobial supplement (MOX, Difco Laboratories, Detroit, MI, USA) and incubated at 35 °C.

2.4. Primary modeling for \( L. \) monocytogenes

The obtained data was applied to the Baranyi (Baranyi & Roberts, 1994; Baranyi, Robinson, Kaloti, & Mackey, 1995), modified Gompertz (Zwietering, Jongenburger, Rombouts, & Van’t Riet, 1990), and Logistic models (Zwietering et al., 1990) for primary modeling. The growth parameters including lag time (LT), maximum specific growth rate (\( \mu_{\text{max}} \)), and maximum bacterial cell density (\( Y_{\text{max}} \)) in the primary model were determined at each temperature with various models using Microfit version 1.0 (Advanced and Hygienic Food Manufacturing LINK Program, UK). The re-parameterized models are described by the following equations (1)–(5).

The Baranyi model:

\[
y(t) = y_0 + \mu_{\text{max}} A(t) - \ln \left( 1 + \left( \frac{\mu_{\text{max}} A(t)}{e^{y_{\text{max}} - y_0}} \right) \right)
\]

where \( A(t) = t + \frac{1}{\mu_{\text{max}}} \ln \left( \frac{e^{\mu_{\text{max}} t + q_0}}{1 + q_0} \right) \)

\[
\text{LT} = \frac{n_1}{n_2}
\]

The modified Gompertz model:

\[
Y = Y_0 + (Y_{\text{max}} - Y_0) \exp \{- \exp[-\mu(t - m)]\}
\]

where \( \mu_\) the relative growth rate growth rate at time \( t = M \)

The Logistic model:

\[
Y = Y_0 + \frac{(Y_{\text{max}} - Y_0)}{1 + \exp(-\mu_0(t - m))}
\]

where \( \mu_\) the relative growth rate at time \( t = M \)

2.5. Secondary modeling

The polynomial model equation was applied for secondary modeling using LT, temperature, and maximum specific growth rate (SCR) (Cho et al., 2011). The equations were described in the following form (6) and (7). The values of \( a, b, \) and \( c \) are constant, and \( T \) is temperature.

\[ y(t) = y_0 + \mu_{\text{max}} A(t) - \ln \left( 1 + \left( \frac{\mu_{\text{max}} A(t)}{e^{y_{\text{max}} - y_0}} \right) \right) \]

\[ A(t) = t + \frac{1}{\mu_{\text{max}}} \ln \left( \frac{e^{\mu_{\text{max}} t + q_0}}{1 + q_0} \right) \]

\[ \text{LT} = \frac{n_1}{n_2} \]

\[ Y = Y_0 + (Y_{\text{max}} - Y_0) \exp \{- \exp[-\mu(t - m)]\} \]

\[ Y = Y_0 + \frac{(Y_{\text{max}} - Y_0)}{1 + \exp(-\mu_0(t - m))} \]
\[
\ln(\text{LT}) = a + bT + cT^2
\]
\[
\ln(\text{SGR}) = a + bT + cT^2
\]

2.6. Evaluation of predictive models

To evaluate the performance of the predicted models, the coefficient of determination \( R^2 \), modified bias factor \( B_f \), accuracy factor \( A_f \), and mean square error (MSE) were used (Abou-zeid et al., 2009; Duffy, Sheridan, Buchanan, McDowell, & Blair, 1994; Ross, 1996; Sutherland, Bayliss, & Robert, 1994).

\[
R^2 = 1 - \left( \frac{\sum e_i^2}{\sum (y_1 - \bar{y})^2} \right)
\]

where \( e_i \): the error of predictive data
\( y_1 \): the predictive data
\( \bar{y} \): the average of predictive data

2.7. Statistical analysis

Culture of \( L. \) monocytogenes were assigned to treatments in triplicate and analysis of variance (ANOVA) was performed on experimental data using SPSS software 8.0 (Statistical Package for the Social Sciences). A significant difference was defined as \( p < 0.05 \).

3. Results and discussion

3.1. Conditions of pork samples

In this study, primary models were developed based on certain condition of pork meat. Microbial counts, pH, and Aw were determined as background information of samples. From Table 1, it appeared that an average total cell number in raw pork samples

Table 1
Conditions of raw pork sample used in this study.

<table>
<thead>
<tr>
<th>Properties</th>
<th>Values (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total aerobic mesophile heterotrophic</td>
<td>4.31 ± 0.02</td>
</tr>
<tr>
<td>bacteria (^a)</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>5.78 ± 0.02</td>
</tr>
<tr>
<td>Water activity</td>
<td>0.997 ± 0.001</td>
</tr>
</tbody>
</table>

\(^a\) The samples were incubated at 35 °C.

Table 2
Growth parameters and evaluations of models (Baranyi, modified Gompertz, and Logistic) to predict the growth of \( L. \) monocytogenes.

<table>
<thead>
<tr>
<th>Model</th>
<th>Temp. (°C)</th>
<th>Growth parameters (mean ± SD)</th>
<th>Evaluations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( y_0 ) (^b)</td>
<td>( y_{\text{max}} ) (^c)</td>
</tr>
<tr>
<td>Baranyi</td>
<td>5</td>
<td>3.05 ± 0.05</td>
<td>6.02 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>3.11 ± 0.00</td>
<td>6.56 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>3.08 ± 0.00</td>
<td>7.27 ± 0.00</td>
</tr>
<tr>
<td>Modified Gompertz</td>
<td>5</td>
<td>3.03 ± 0.03</td>
<td>5.98 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>3.07 ± 0.04</td>
<td>6.52 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>3.02 ± 0.06</td>
<td>7.22 ± 0.04</td>
</tr>
<tr>
<td>Logistic</td>
<td>5</td>
<td>3.06 ± 0.01</td>
<td>6.01 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>3.12 ± 0.02</td>
<td>6.55 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>3.12 ± 0.03</td>
<td>7.25 ± 0.02</td>
</tr>
</tbody>
</table>

\(^a\) \( y_0 \): initial cell count (log CFU/g).
\(^b\) \( y_{\text{max}} \): maximum cell count (log CFU/g).
\(^c\) \( m_{\text{max}} \): maximum specific growth rate (log CFU/g/h).
\(^d\) LT: lag time (h).

Fig. 1. The growth of \( L. \) monocytogenes on raw pork at various storage temperatures (●: 5 °C, ■: 15 °C, ▲: 25 °C).
was 4.31 log CFU/g. The pH of sample was 5.78 and Aw was 0.997. The samples used in this work expressed similar value with previous study. Many researchers have reported that *L. monocytogenes* can develop at low temperatures in a wide pH and with a high concentration of salts, and, hence, it can survive and multiply in a great variety of food products (Begot, Lebert, & Lebert, 1997; Cole, Jones, & Holyoak, 1990; Karina, Julio, Leda, & Nemi, 2011).

### 3.2. Growth of *L. monocytogenes* on raw pork

Table 2 and Fig. 1 show that *L. monocytogenes* grows well on raw pork from 5 °C to 25 °C. The initial population density was between 3.04 and 3.09 log CFU/g, showing that it was not significantly different. The maximum population density was between 6.02 and 7.27 log CFU/g at 5 °C–25 °C, respectively. Capozzi, Fiocco, Amodio, Gallone, and Spano (2009) and Lebert, Robles-Olvera, and Lebert (2000) determined that the differences in maximum population density were due to the background levels of the microflora. In this study, the samples were not sterilized; such variable micro-flora may explain the interrupted growth of *L. monocytogenes*. The growth of on the raw pork stored at 5 °C did not occur until 174 h. This result was some difference from the results of Hong et al. (2005): the lag time was reported to be 43.3 h at 5 °C on the raw pork. However, Amit, Theodore, and Francisco (2008) reported that the growth of *L. monocytogenes* was different according to Listeria strains and that maximum lag time among the strains was 4 days at temperature range of 4–12 °C. As storage temperature increased, the lag time in this study decreased with values of 174 h (5 °C), 7.27 h (15 °C), and 3.99 h (25 °C). In contrast, the specific growth rate (μ_max) increased gradually with values of 0.05 (5 °C), 0.47 (15 °C), and 0.65 (25 °C) log CFU/g/h as the storage temperature increased.

Both this study and previous other studies have demonstrated that *L. monocytogenes* can grow at refrigeration temperatures of 10 °C or less, but growth was reduced at temperatures below 0 °C. Therefore, these data emphasize the importance of low temperature during manufacturing processing steps for microbiological safety, hygienic handling, and management of the distribution process (Hong et al., 2005).

### 3.3. Development of growth models for *L. monocytogenes*

Predictive models in this study were developed by the Baranyi, modified Gompertz, and Logistic models based on experimental data and were shown in Table 2. The Baranyi model predicted that the specific growth rate (μ_max) increased gradually with values of 0.05, 0.47, and 0.65 log CFU/g/h as storage temperatures increased (5, 15, and 25 °C, respectively). However, the specific growth rates in the modified Gompertz and Logistic models were higher than the rates predicted in the Baranyi model. The modified Gompertz model predicted that the specific growth rates at 5, 15, and 25 °C were 0.11, 1.01, and 1.33 log CFU/g/h. In the Logistic model, the specific growth rates at 5, 15, and 25 °C were predicted to be 0.11, 0.97, and 1.29 log CFU/g/h. Park, Bahk, Park, Park, and Ryu (2010) showed similar results where the specific growth rate using the Baranyi model was greater than the rate using the Gompertz model.

The modified Gompertz and Logistic models represent empirical models, which have been widely used in the literature. However, these methods occasionally overestimate the specific growth rate of microorganisms during culturing (Ye et al., 2013). In this study, the values of Y0, μ_max, and LT were not significantly different between each model.

### 3.4. Evaluation of predictive models

Table 2 shows the correlation of the predicted values obtained by the secondary developed model with the values observed in this study. The mathematically predicted growth rate parameters were evaluated using the bias factor (B_f), accuracy factor (A_f), and mean square error (MSE). At all of the storage temperatures (5, 15, and 25 °C), MSE values used in the Baranyi model were 0.006, 0.006, and 0.011, respectively, which were lower than those obtained in the other models. The low MSE value suggested that the model was reasonably accurate in describing the effect of time on colony formation of *L. monocytogenes* (Zwietering et al., 1990). The values of B_f and A_f from all the models were near 1.0.

Fig. 2 shows the coefficient of determination (R^2) used in the Baranyi model. R^2 values in all the models at 5, 15, and 25 °C were
estimated to be over 0.9 (data not shown), and those of the Baranyi model were more appropriate than that for any other model. At each storage temperature (5, 15, and 25 °C), $R^2$ values were 0.9961, 0.9978, and 0.9975, respectively. Therefore, these data indicate that the Baranyi model provided a good statistical fit to the observed data, and the $R^2$ values were near 1.0. The Baranyi model has been shown to be more suitable in other studies. For example, Ye et al. (2013) calculated $R^2$ values by using the Baranyi model and they obtained 0.9752, 0.9965, 0.9990, 0.9993, and 0.9995 at various storage temperatures (4, 10, 15, 20, and 25 °C, respectively) in vacuum-packaged chilled pork (Thomas & Matthews, 2005, pp. 247–250; Ye et al., 2013).

3.5. Secondary modeling and validation

The predictive data from the Baranyi model, which was the most accurate of the three models, was used for secondary modeling. Secondary modeling was developed using equations (6) and (7). Fig. 2 shows the comparison of the linear regression models for LT and $\mu_{max}$. As the storage temperature increased (5, 15, and 25 °C), the Baranyi model predicted that $\mu_{max}$ increased gradually with the values of 0.05, 0.47, and 0.65 CFU/g/h, respectively, while ln(LT) decreased with values of 2.242, 0.861, and 0.601, respectively. It appeared that the values of $\mu_{max}$ were inversely proportional to ln(LT). These data demonstrate that the growth of microorganisms can be dependent on storage temperature. It has been reported that temperature is an important environmental parameter affecting microbial growth and spoilage of meat or meat products (Sant’Ana, Landgraf, Destro, & Franco, 2011). $\mu_{max}$ value was characteristic of microbial growth in a particular environment, while LT was influenced by the history and the physiological state of bacterial cells (Baranyi et al., 1995).

Table 3 shows validation of secondary model to predict the growth of *L. monocytogenes* on raw pork. For the SGR model, both $B_f$ and $A_f$ were 1.000. It meant that observed value and predicted value were absolutely identical. In the ln(LT) model, $B_f$ and $A_f$ were 0.962 and 1.041, respectively. Thus, the results showed that both the $B_f$ and $A_f$ values of ln (LT) and SGR models were close to 1. The MSE values of the SGR model and ln(LT) model are 0.000 and 0.056, respectively. Based on Table 3, it was presumed that the polynomial model equation which was used to develop models can present the growth of *L. monocytogenes* on raw pork.

For ComBase program and the pathogen modeling program (PMP), pH and Aw conditions in Table 1 were used. Fig. 3 shows that the pattern of the secondary model derived from ComBase was similar to that derived from PMP regarding LT and $\mu_{max}$. However, $\mu_{max}$ calculated from the models developed in this study for *L. monocytogenes* was slightly different from those of both ComBase and PMP, which may be due to the use of different culture media between each experiment. Microorganisms were not exposed to factors such as the structure and components of foods and background microorganisms when they are grown in culture media (Maarten, 2012).

### Table 3

<table>
<thead>
<tr>
<th>Polynomial model equation</th>
<th>ln (LT or SGR) = a + b × T + c × T²</th>
<th>MSEa</th>
<th>$B_f$</th>
<th>$A_f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGR = 3.3603 – 0.2515 × T + 0.0056 × T²</td>
<td>0.000</td>
<td>1.000</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>ln (LT) = –0.25 + 0.0067 × T – 0.0012 × T²</td>
<td>0.056</td>
<td>0.962</td>
<td>1.041</td>
<td></td>
</tr>
</tbody>
</table>

a: MSE: mean square error.
b: $B_f$: bias factor.
c: $A_f$: accuracy factor.

4. Conclusion

The purpose of this study was to develop a predictive model of *L. monocytogenes* for effective hygiene management and growth patterns at refrigeration temperatures by using a strain isolated from pork. The results of this study showed that the Baranyi model was more suitable than the modified Gompertz or Logistic models for predicting the growth of *L. monocytogenes* on raw pork.

A predictive model has recently been developed in an inoculate-pack study of food products, as opposed to using a culture medium (Maarten, 2012). In this study, a predictive model was developed by changing the temperature and time. Although the pH and water activity ($A_w$) of raw pork can affect microbial growth, these factors have little effect on the quality of the meat until it is consumed (Joo, Kim, Hwang, & Ryu, 2013; Kim et al., 1997). Therefore, these were not considered as major factors for predictive modeling in this study. In addition, the initial number of cells inoculated onto the pork samples was higher than the actual levels of contamination in commercial food products, but it has been reported that the initial cell number does not affect the development of predictive models (Duffy et al., 1994; Hong et al., 2005).

The predictive model in this study can predict the growth of foodborne pathogens with different transitions of time and temperature. Therefore, this model could be used to set up the critical control points (CCP) on storage temperature as HACCP in meat industrial processing and distribution to improve food safety (Bahk et al., 2001).