Growth kinetics of Escherichia coli O157:H7 in mechanically-tenderized beef

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A kinetic study was conducted to investigate the growth of Escherichia coli O157:H7 in mechanically-tenderized beef meat (MTBM) inoculated and internalized with a cocktail of rifampicin-resistant (Rifr) or 3 arbitrarily selected wild-type strains of the bacteria. The storage was conducted at 5, 10, 15, 20, 25, and 37 °C. No growth was observed at 5 °C and the growth was minimal at 10 °C. Above 15 °C, a sigmoid trend was observed for all growth curves. Three primary growth models (modified Gompertz, Huang, and Baranyi) were used to fit the growth curves. A new Belehradek-type secondary model was found more suitable than the traditional Ratkowsky model for describing the temperature dependence of growth rate. The statistical analysis suggested that both bacterial strains and primary growth model affect the determination of growth rates (at α = 0.05), with Rifr strains growing 10–20% slower than the wild-type strains. While there was no significant difference between the growth rates estimated by the modified Gompertz and Baranyi models, and between those of Huang and Baranyi models, the rates estimated from the Gompertz model were significantly higher than those estimated from the Huang model.

The temperature dependence of growth rate of E. coli O157:H7 in MTBM was described by both a new Belehradek-type rate model and the Ratkowsky square-root model. While the theoretical minimum growth temperatures determined by the Ratkowsky square-root model ranged from 1.5 to 4.7 °C, more realistic values, varying from 6.6 to 8.76 °C, were estimated by the new rate model. For the Baranyi model, the average h0 value was 2.06 ± 0.74 and 2.15 ± 1.14 for Rifr and wild-type strains of E. coli O157:H7, respectively. For the Huang and modified Gompertz models, the inverse of square-roots of lag phases was found proportional to temperature, making it possible to estimate the lag phase duration from the growth temperature. The results of this work can be used to assess the microbial safety of MTBM during refrigerated and temperature-abused storage conditions.

1. Introduction

Enterohemorrhagic Escherichia coli O157:H7 is one of the most deadly foodborne pathogens that have become one of the most significant public health hazards in the United States and around the world. Recognized as a foodborne pathogen since 1980s (Riley et al., 1983; Doyle and Schoeni, 1984), E. coli O157:H7 has caused outbreaks linking to the consumption of a variety of unpasteurized, uncooked, or undercooked foods, including ground beef, raw milk and dairy products, and vegetables. Raw and undercooked foods of bovine origin, particularly ground beef (Doyle, 1991), are undoubtedly the most important transmission vehicle of E. coli O157:H7 (Hancock et al., 1997; Park et al., 1999; Gansheroff and O’Brien, 2000).

Although various animals can carry E. coli O157:H7, cattle remain the most important reservoir (Buchanan and Doyle, 1997; Gansheroff and O’Brien, 2000). This microorganism colonizes the gastrointestinal tract of cattle and is shed in the feces of the animals (Reinstein et al., 2007). Fecal contamination, which occurs during the removal of hides and hairs (Buchanan and Doyle, 1997), is a major route of transmission that contributes to foodborne infections associated with E. coli O157:H7. While structurally-intact beef steaks are rarely linked to E. coli O157:H7 infection, contaminated meat, when made into ground beef, can cause large-scale outbreaks.

Mechanically-tenderized beef meat (MTBM) is a value-added product made from sub-primal beef cuts. During mechanical tenderization, mechanical blades are inserted into sub-primal cuts, disrupting the structure of beef muscle (Sutterfield, 2007). During this process, bacteria contaminated on the surfaces of beef cuts are carried and translocated into the interior, leading to internal contamination. According to Sporing (1999), approximately 3–4% of E. coli O157:H7 inoculated onto meat surfaces can be translocated internally. Other studies (Luchansky et al., 2008, 2009; Echeverry et al., 2009) also reported internal translocation of E. coli O157:H7 cells after blade tenderization. Decontamination of sub-primal beef cuts prior to

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mechanical tenderization did not seem to be very effective in reducing the surface bacterial concentration (Heller et al., 2007). Outbreaks of *E. coli* O157:H7 infections and recalls associated with non-intact blade-tenderized beef products have been reported (Laine et al., 2005; USDA FSIS, 2002, 2005). Since 1999, USDA FSIS began to expand the *E. coli* O157:H7 adulteration policy to include non-intact beef products (USDA FSIS, 2005). Under this policy, non-intact beef products, if found contaminated with *E. coli* O157:H7, must be processed into ready-to-eat products, or they would be considered adulterated.

For contaminated MTBM, the fate of *E. coli* O157:H7 during storage has not been studied and this information is necessary for risk assessment. Therefore, the main objective of this study is to investigate the fate of *E. coli* O157:H7 in MTBM and develop kinetic models to describe its growth during storage at selected temperature conditions. In the previous blade tenderization studies (Sporing, 1999; Phebus et al., 2000; Luchansky et al., 2008, 2009), *E. coli* O157:H7 strains resistant to rifampicin were used in the translocation investigations. While using antibiotic-resistant strains makes it easier to differentiate them from background bacterial flora, it is not clear whether the growth behavior of these strains is modified by the antibiotic. Therefore, another objective of this study is to evaluate the suitability of the rifampicin-resistant (Rif^r^) strains of *E. coli* O157:H7 for kinetic studies and compare its growth kinetics with arbitrarily selected strains of *E. coli* O157:H7. The resulting knowledge of growth kinetics of *E. coli* O157:H7 in MTBM would lead to better understanding of survivability and multiplication of this pathogen under various natural temperature conditions.

2. Materials and methods

2.1. Rif^r^ strains of *E. coli* O157:H7

Five Rif^r^ strains of *E. coli* O157:H7, identical to the ones used in Luchansky et al. (2008, 2009), were used in this study. The five Rif^r^ *E. coli* O157:H7 were 1) USDA/FSIS 011-82; 2) ATCC 43888; 3) ATCC 43889; 4) ATCC 43890; and 5) USDA-FSIS 45756. The bacterial cultures were stored in a refrigerator on individual Sorbitol-MacConkey Agar (SMAC, BD, Sparks, MD) plates supplemented with 100 µg/ml rifampicin (R-7382, Sigma, St. Louis, MO), or SMAC-R plates. The cultures were regularly (every 2–3 weeks) transferred to maintain the viability of cells. Using SMAC-R plates allowed exclusive recovery of Rif^r^ strains of *E. coli* O157:H7 from MTBM, without interference from any background bacterial flora.

2.2. Wild-type strains of *E. coli* O157:H7

Three wild-type outbreak strains of *E. coli* O157:H7, previously isolated from bovine sources and arbitrarily selected from the culture collection maintained at the USDA ARS Eastern Regional Research Center, Wyndmoor, PA, were also used in this study. These strains included 1) USDA/FSIS 45753-35; 2) *E. coli* O157:H7 strain 933; and 3) *E. coli* O157:H7 strain A9218-C1. These strains were stored in a refrigerator on SMAC Agar supplemented with Cefixime and Tellurite (BD, Sparks, MD), or SMAC-CT plates. The cultures were also regularly transferred to maintain the viability of cells. While less selective than SMAC-R plates, SMAC-CT plates were more selective than original SMAC plates by suppressing more background bacterial flora. Therefore, SMAC-CT plates allowed selective recovery of wild strains of *E. coli* O157:H7 from raw MTBM. Using SMAC-CT plates allowed comparing the growth kinetics of wild-type strains of *E. coli* O157:H7 with that of Rif^r^ strains.

2.3. Culture preparation

To grow the cultures for inoculation, a loopful of an isolated colony on SMAC-R or SMAC-CT plates from each strain was transferred to 10 ml Brain Heart Infusion Broth (BHI, BD, Sparks, MD), and incubated at 37 °C overnight with mild shaking. Each culture was harvested by centrifugation (2400 g for 10 min at 4 °C), washed once and re-suspended with 5 ml 0.1% peptone water (PW, BD, Sparks, MD). A cocktail of Rif^r^ strains of *E. coli* O157:H7 cells was formed by combining the five Rif^r^ cultures. A cocktail of wild-type cells was formed by mixing the wild-type cultures. The cocktails were immediately used to inoculate beef samples after proper dilution.

2.4. Sample preparation and inoculation

Previous studies suggested a wide range of distributions of *E. coli* O157:H7 in different locations and even on the surface layers of MTBM (Luchansky et al., 2008, 2009). The samples collected from these experiments were not suitable for developing growth models, which would require relatively uniform distribution of bacterial cells. As the majority of the translocated bacterial cells was distributed on the top layer (Luchansky et al., 2008, 2009), the following procedure was adopted to prepare MTBM samples.

Beef (bottom round roast) meat, purchased from a local butcher shop, was tenderized with a handheld meat tenderizer (Model MT-48, Keystone Manufacturing Co, Inc., Buffalo, NY) with needle configurations identical to commercial blade tenderizers. Meat samples were tenderized in a direction perpendicular to muscle fibers. The blade-tenderized meat was cut into 5 g portions, along the direction of muscle fibers, approximately 5 cm in thickness. Each sample was transferred to a filter bag (Whirl-Pak®, 7 oz, 95 mm x 180 mm x 0.08 mm, NASCO — Fort Atkinson, Fort Atkinson, WI).

Each sample was inoculated with 0.1 ml of with either Rif^r^ or wild-type bacterial cocktail solution. Each sample was briefly (~5 s) palpitated in the filter bag to internalize the inoculated bacteria using a mechanical stomacher (Model BagMixer® 100 W, Interscience Co., France). The concentration of bacteria was approximately 1000 cells/g, or approximately 3 log_{10} cfu/g. Since the bacteria were suspended in PW, they could move freely into the holes punctured by the needle tenderizer during mechanical palpitation in the stomacher. After inoculation, the opening of each filter bag was loosely closed with the attached metal wire to prevent contamination.

2.5. Storage and growth study

Inoculated beef samples were incubated isothermally at 5, 10, 15, 20, 25, and 37 °C for different lengths of time, depending on incubation temperature. Samples were periodically retrieved for enumeration of bacterial cells. The growth studies at 5, 10, 15, and 20 °C were conducted in duplicate. At 25 and 37 °C, the growth studies were replicated at least three times.

2.6. Enumeration of bacteria

After incubation, samples were added with 20 ml ice-cold PW and blended in the previously mentioned stomacher at the maximum speed setting for 6 min. The filtered liquid portion was withdrawn and serially diluted for enumerating *E. coli* O157:H7 cells. For Rif^r^ strains, the bacterial cells were surface-plated onto to SMAC-R agar plates. For wild-type strains, the bacterial cells were surface-plated onto SMAC-CT agar plates (Tamplin, 2002). The SMAC plates were incubated in a 37 °C incubator overnight. Typical colonies of *E. coli* O157:H7 on SMAC plates were counted and converted to natural logarithm of colony forming unit (cfu) per unit gram of beef meat, or ln cfu/g, which is 2.303 log_{10} cfu/g.

2.7. Growth kinetics and mathematical modeling

Three growth models were chosen as primary models. The first model was the modified Gompertz model. Although this model is only
an empirical model and can overestimate the maximum growth rate at times, it is a model that has been wildly used in the literature. A large body of kinetic data was derived from this model. Therefore, this model was used a reference model. To directly compare this model with two other more biologically-based growth models using in this study, however, the natural logarithm of bacterial counts, instead of the logarithm of base, was directly used:

\[ Y = Y_0 + (Y_{max} - Y_0) \exp\{ -\exp[-\mu_c(t-M)] \} \]  

In Eq. (1), \( Y \) is the natural logarithm of bacteria count at any given time, \( \ln \text{cfu/g} \); \( Y_{max} \) and \( Y_0 \) are the maximum and initial values of \( Y \); \( \mu_c \) is the relative growth rate at time = \( M \), which is the inflection point of the curve. From Eq. (1), two parameters, lag phase (\( \lambda \)) and specific growth rate (\( K \)), pertinent to bacterial growth under constant temperature conditions can be derived.

\[ \lambda = M - \frac{1}{\mu_c} \]  

\[ K = \frac{(Y_{max} - Y_0)\mu_c}{e} \]  

The second growth model was modified from Huang (2008). This model was derived from the three phase (lag, exponential, and stationary) growth phenomenon under isothermal conditions, and is written as

\[ Y = Y_0 + Y_{max} - \ln \{ \exp(Y_0) + \exp(Y_{max}) - \exp(Y_0) \} \exp[-K \times B(t)] \]  

where,

\[ B(t) = t + \frac{1}{25} \ln \frac{1 + \exp[-25(t-\lambda)]}{1 + \exp(25\lambda)} \]  

2.8. Effect of temperature on growth rates

The traditional Ratkowsky square-root model (Ratkowsky et al., 1982), Eq. (7), is probably the most widely used secondary model to evaluate the effect of temperature on growth of bacteria. This model required the transformation of \( K \) and was analyzed using the NLIN procedure of the statistical package SAS (Version 9.5, Cary, NC) was used to obtain the estimates of \( a \) and \( T_{min} \).

\[ \sqrt{K} = a(T - T_{min}) \]  

The mean square error (MSE), generated by SAS, was used to evaluate the performance of the Ratkowsky model. The MSE of a model was calculated by

\[ MSE = \frac{\sum_{i=1}^{n} (y_i - \hat{y}_i)^2}{df} \]  

In Eq. (8), \( n \) is the number of observations; \( df \) is the degree of freedom, or \( n - 2 \); \( y_i \) is the ith observed value; and \( \hat{y}_i \) is the estimated value for the ith observation.

2.9. Statistical analysis

Analysis of variance (ANOVA) was used to compare the difference between the growth kinetics of Rif+ and wild-type E. coli O157:H7.

![Fig. 1. Growth of Rif+ and wild strains of E. coli O157:H7 in MTBM at 5 and 10 °C.](image-url)
The statistical analysis was conducted using SAS (Version 9.5, Cary, NC). The difference in the growth rate \( (K) \) derived from the three models was also analyzed by ANOVA. If significant difference was observed \( (P<0.05) \), the Tukey’s Studentized Range (HSD) test procedure was used to compare and group the means of growth rates.

3. Results and discussion

3.1. Observation and mathematical modeling of isothermal growth curves

MTBM samples inoculated with Rif\( ^+ \) and wild-type strains of \textit{E. coli} O157:H7 were incubated at 5, 10, 15, 20, 25, and 37 \(^\circ\)C. Since raw beef

Fig. 2. Growth of Rif\( ^+ \) and wild strains of \textit{E. coli} O157:H7 in MTBM at 15 and 20 \(^\circ\)C. The empty squares represent the raw data points; the thin solid curves represent the modified Gompertz model; the thick solid curves represent the Huang model; and the dashed curves represent the Baranyi model.

Fig. 3. Growth of Rif\( ^+ \) and wild strains of \textit{E. coli} O157:H7 in MTBM at 25 and 37 \(^\circ\)C. The empty squares represent the raw data points; the thin solid curves represent the modified Gompertz model; the thick solid curves represent the Huang model; and the dashed curves represent the Baranyi model.
samples were used, the inoculated \( E. \) \( \text{coli} \) O157:H7 cells had to compete with the background bacterial flora for survival. Even with the presence of competing background bacterial flora, the \( E. \) \( \text{coli} \) O157: H7 colonies were easily identified by the use of selective media (SMAC-CT). For Rif\(^{\text{r}}\) strains of \( E. \) \( \text{coli} \) O157:H7 cells recovered from samples, the background cells were suppressed by rifampicin added to SMAC agar, and only Rif\(^{\text{r}}\) \( E. \) \( \text{coli} \) O157:H7 colonies emerged from SMAC-R plates. For wild-type strains of \( E. \) \( \text{coli} \) O157:H7 plated onto SMAC-CT agar plates, some colonies of background bacterial flora also grew, but the colorless colonies of \( E. \) \( \text{coli} \) O157:H7 were clearly identifiable and easily distinguished from the remaining background bacterial flora.

According to experimental observations, both Rif\(^{\text{r}}\) and wild-type strains of \( E. \) \( \text{coli} \) O157:H7 did not grow well at 5 and 10 °C (Fig. 1). At 5 °C, no growth of \( E. \) \( \text{coli} \) O157:H7 cells was observed (Fig. 1), and the number of bacteria actually declined. At 10 °C, minimum growth (<1 log cfu/g) was observed for up to 25 days. At these low incubation temperatures (5 and 10 °C), the background bacterial flora, particularly psychrotrophic bacteria, thrived. \( E. \) \( \text{coli} \) O157:H7, however, is a mesophilic bacterium. Therefore, \( E. \) \( \text{coli} \) O157:H7 incubated at these refrigerated temperatures was generally out-competed by background bacterial flora and was prevented from continued growth. A distinct rotten odor was produced as storage progressed, which may become a warning sign for consumers. However, the \( E. \) \( \text{coli} \) O157:H7 cells survived well during refrigerated storage 5 °C. The growth at 10 °C did not reach its full potential for both Rif\(^{\text{r}}\) and wild-type strains of \( E. \) \( \text{coli} \) O157:H7.

\( E. \) \( \text{coli} \) O157:H7 grew better at temperatures above 15 °C, and all growth curves exhibited lag, exponential, and stationary phases (Figs. 2 and 3). The duration of lag phase and exponential growth rate was clearly affected by temperature, but the concentration of stationary phase cells was between 8.1 and 8.2 log\(_{10}\) cfu/g or 18.7 Ln cfu/g, not affected by temperature.

All three models, modified Gompertz, Huang, and Baranyi models, were suitable to describing the growth of \( E. \) \( \text{coli} \) O157:H7 cells inoculated into MTBM and incubated at 15, 20, 25, and 37 °C (Figs. 2 and 3). Compared with modified Gompertz and Baranyi models, the Huang model has a more clearly identifiable lag phase. The modified Gompertz model and the Baranyi model resemble more with each other in the lag phase. The Huang and Baranyi models resemble one another in the exponential and stationary phases since both models use logistic competitive growth scheme in the exponential and stationary phases.

### 3.2. Effect of temperature on growth rate and comparison of primary models

The effect of storage temperature on the growth rate \( E. \) \( \text{coli} \) O157: H7 at temperatures above 10 °C was evaluated by the traditional Ratkowsky square-root model, and Table 1 lists the result of nonlinear analysis of the growth rates of Rif\(^{\text{r}}\) and wild-type \( E. \) \( \text{coli} \) O157:H7 derived from the three primary models. The estimated value of coefficient \( a \) for the square-root model ranged from 0.0334 to 0.0420 with relatively small standard errors. It is noticeable that the estimate of the minimum growth temperature was remarkably similar for Rif\(^{\text{r}}\) or wild-type strains of \( E. \) \( \text{coli} \) O157:H7. The minimum growth temperature (\( T_{\text{min}} \)) estimated by the traditional Ratkowsky square-root model ranged from 1.5 to 4.7 °C. Also according to Table 1, the mean square of the model, which is related to the sum of squares associated with the model, was between 1.2 and 1.9, while the mean square error (MSE) of the Ratkowsky model was relatively small, ranging from 0.002 to 0.009. The small MSE values suggest that the model was reasonably accurate describing the effect of temperature on the square-root of specific growth rates (Fig. 4).

The parameter \( T_{\text{min}} \) in the Ratkowsky square-root model was the theoretical minimum temperature that the bacteria can grow. Regardless of the growth models used to determine growth rate in this study, the values of \( T_{\text{min}} \) ranged from 1.5 °C to 4.7 °C, depending on the primary models and the type (Rifr or wild) of \( E. \) \( \text{coli} \) O157:H7.

A range of theoretical minimum growth temperature (275, 276, 277, or 280 K) of \( E. \) \( \text{coli} \) was listed in Ratkowsky et al. (1982), depending on

### Table 1 Parameters for the Ratkowsky square-root model (Eq. (7)): \( \sqrt{K} = a(T - T_{\text{min}}) \).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Modified Gompertz</th>
<th>Huang</th>
<th>Baranyi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rif(^{\text{r}})</td>
<td>Wild</td>
<td>Rif(^{\text{r}})</td>
</tr>
<tr>
<td>( a )</td>
<td>0.0380(^{a})</td>
<td>0.0420</td>
<td>0.0362</td>
</tr>
<tr>
<td>( T_{\text{min}} ) (°C)</td>
<td>(0.0028(^{b}))</td>
<td>(0.0023)</td>
<td>(0.0023)</td>
</tr>
<tr>
<td></td>
<td>3.81(^{a})</td>
<td>4.66</td>
<td>4.10</td>
</tr>
<tr>
<td></td>
<td>(1.59(^{b}))</td>
<td>(1.11)</td>
<td>(1.33)</td>
</tr>
<tr>
<td>Mean square error</td>
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<td>0.006</td>
<td>0.006</td>
</tr>
<tr>
<td>Model mean square</td>
<td>1.572</td>
<td>1.915</td>
<td>1.424</td>
</tr>
</tbody>
</table>

\(^{a}\) Estimated value of a parameter (\( a \) or \( T_{\text{min}} \)).

\(^{b}\) Approximated standard error.

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Fig. 4. Ratkowsky model used to describe the temperature dependence of specific growth rate of Rif\(^{\text{r}}\) and wild strains of \( E. \) \( \text{coli} \) O157:H7 in MTBM, derived from the modified Gompertz, Huang, and Baranyi models.
the source of the data. The theoretical minimum growth temperature obtained from this study was very close to the values listed in Ratkowsky et al. (1982).

However, *E. coli* O157:H7 is a mesophilic bacterium. It cannot grow well at these temperatures. The estimated $T_{\text{min}}$ determined from the Ratkowsky square-root models was too low for *E. coli* O157:H7. It was observed in this study that the population of *E. coli* O157:H7 in MTBM actually declined during refrigerated storage at 5 °C, although they could survive the refrigerating temperature. According to Buchanan and Bagi (1994), Buchanan and Doyle (1997), and Rajkowski and Marmer (1995), the minimum growth temperature for *E. coli* O157:H7 under optimal conditions is approximately 8–10 °C. In the study conducted by Palumbo et al. (1995), most tested *E. coli* O157:H7 strains started to grow at 10 °C in BHI broth, but not at 8 °C. Tamplin (2002) studied the growth of *E. coli* O157:H7 in raw ground beef at 10 °C and reported a growth rate at this temperature ranging from 0.009 to 0.025 log$_{10}$ cfu/g/h (mean = 0.019 log$_{10}$ cfu/g/h), which is very close to the growth rate (0.01–0.02 log$_{10}$ cfu/g/h) observed at 10 °C in MTBM in this study. Massa et al. (1999) investigated the fate of 7 strains of *E. coli* O157:H7 in unpasteurized milk stored at 8 °C, and reported that there was

Table 2

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Modified Gompertz</th>
<th>Huang</th>
<th>Baranyi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rif$^*$</td>
<td>Rif$^*$</td>
<td>Rif$^*$</td>
</tr>
<tr>
<td>$a$</td>
<td>0.00897$^a$</td>
<td>0.0118</td>
<td>0.00859</td>
</tr>
<tr>
<td></td>
<td>(0.00092$^b$)</td>
<td>(0.0014)</td>
<td>(0.00072)</td>
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<tr>
<td>$T_{\text{min}}$ (°C)</td>
<td>6.64$^a$</td>
<td>8.57</td>
<td>7.77</td>
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<td></td>
<td>(1.58$^b$)</td>
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<td>Mean square error</td>
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<td>Model mean square</td>
<td>4.608</td>
<td>6.237</td>
<td>3.677</td>
</tr>
</tbody>
</table>

$^a$ Estimated value of a parameter ($a$ or $T_{\text{min}}$).

$^b$ Approximated standard error.

Fig. 5. The new rate model used to describe the temperature dependence of specific growth rate of Rif$^*$ and wild strains of *E. coli* O157:H7 in MTBM, derived from the modified Gompertz, Huang, and Baranyi models. The solid lines represent estimated values and the dotted lines represent upper and lower 95% confidence intervals.
essentially no change in the viable population in 3 strains for up to 14 days, while the growth of the other 4 strains was less than 2–3 log cfu/ml between 7th and 17th day. It is apparent that E. coli O157:H7 cells does not grow well at temperature below 10 °C. Apparently, the minimum growth temperature estimated by the Ratkowsky square-root model in this study apparently did not match well with the biological nature of E. coli O157:H7.

Two recent studies also have reported similar disparity between the minimum growth temperature and T_{min} estimated from the Ratkowsky square-root model. Dominguez and Schaffner (2008) conducted a study to investigate the growth kinetics of Salmonella, also a mesophilic bacterium, in raw poultry and used the Ratkowsky square-root model to evaluate the effect of temperature on growth rate. They reported a theoretical T_{min} of 4.1 °C, which is also below the minimum growth temperature for mesophilic bacteria. Juneja et al. (2009) studied the growth kinetics of Salmonella in raw ground beef and used four primary growth models, including modified Gompertz, modified logistic, Baranyi, and Huang models to fit the growth curves. The minimum growth temperature (T_{min}) derived from modified Ratkowsky model (K = a(T−T_{min})^{b}[1−\exp(b(T−T_{max}))]) ranged from −0.1 to 0.9 °C, which is similar to the results obtained in this study. It is possible that the square-root model may actually underestimate the minimum growth temperature of mesophilic bacteria.

That the minimum theoretical temperature T_{min} estimated by the traditional or modified Ratkowsky model is not in agreement with the biological nature of mesophilic bacteria suggests that there is a need to use a different secondary model to evaluate the effect of temperature on growth rate, as suggested by Dantigny and Molin (2000). To more accurately reflect the minimum growth temperature of E. coli O157:H7 in MTBM, a modified rate model (Eq. (9)) was used to relate the growth rate data to temperature. This rate model is one of the Belehradek-type models, according to McMeekin et al. (1993). The NLIN procedure in SAS was used to obtain the statistics of a and T_{min}. Table 2 lists the results of statistical analysis (ANOVA) for the determination of growth rate. Table 3 lists the results of statistical analysis (ANOVA) of the effect of temperature, bacterial strain, and primary model on the determination of growth rate. Apparently, the effect of temperature was significant (P=0.0001) on the growth rate since the growth of E. coli O157:H7 in MTBM was highly temperature-dependent.

The choice of primary model affected the determination of growth rate (P=0.0093). According to the results of Tukey's Studentized Range (HSD) test (α = 0.05, data not shown), the mean of growth rates determined from the modified Gompertz model was not significantly different from that determined from the Baranyi model, but is significantly higher than that determined from the Huang model. There was no significant difference between the means of growth rate determined from the Baranyi and Huang models. The growth rate was also affected by the bacterial strain (P=0.0129, Table 3). The mean growth rate of wild-type strains of E. coli O157:H7 documented in the literature, suggesting that the new rate model may be a more suitable secondary model for mesophilic bacteria such as E. coli O157:H7 in meats. It is also noteworthy to point out that the bacteria survive very well at temperatures below the minimum growth during refrigerated storage, and the presence of E. coli O157: H7 always represents potential food safety risks.

### 3.3. Effect of strains of bacteria and primary model on determination of growth rate

Since Rif\(^{+}\) strains of E. coli O157:H7 were used in this study, one question was whether they would grow differently from randomly selected wild-type strains of E. coli O157:H7. Another important question was whether the primary models affected the determination of growth rate. Table 3 lists the results of statistical analysis (ANOVA) of the effect of temperature, bacterial strain, and primary model on the determination of growth rate. Apparently, the effect of temperature was significant (P=0.0001) on the growth rate since the growth of E. coli O157:H7 in MTBM was highly temperature-dependent.

The choice of primary model affected the determination of growth rate (P=0.0093). According to the results of Tukey's Studentized Range (HSD) test (α = 0.05, data not shown), the mean of growth rates determined from the modified Gompertz model was not significantly different from that determined from the Baranyi model, but is significantly higher than that determined from the Huang model. There was no significant difference between the means of growth rate determined from the Baranyi and Huang models. The growth rate was also affected by the bacterial strain (P=0.0129, Table 3). The mean growth rate of wild-type strains of E. coli O157:H7:

\[
K = a(T−T_{min})^{b.5}
\]

According to Table 2, the calculated theoretical minimum growth temperature T_{min} derived from the modified Gompertz, Huang, or Baranyi model, for either Rif\(^{+}\) or wild-type strains was around 6.64–8.76 °C. It is also necessary to note that MSEs listed in Table 1 for the traditional Rajkowsky square model appear smaller than those listed in Table 2. The traditional Rajkowsky square-root model requires transforming of growth rates into their square roots, which in reality leads to smaller calculated MSE in Table 1. The new rate model (Eq. (9)) requires no data transformation. Using the traditional Ratkowsky square-root model would lead to positive growth rates at temperatures well below the biological limits of E. coli O157:H7, thus overestimating the growth at refrigerated temperature ranges (such as at 5 °C).

With the new rate model (Eq. (9)), the estimated minimum theoretical growth temperature (T_{min}) is now more accurately reflecting the true minimum growth temperature for E. coli O157:H7.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>ANOVA SS</th>
<th>Mean square</th>
<th>F value</th>
<th>Pr&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp</td>
<td>4</td>
<td>212.94</td>
<td>53.23</td>
<td>400.66</td>
<td>0.0001</td>
</tr>
<tr>
<td>Model</td>
<td>2</td>
<td>0.137</td>
<td>0.069</td>
<td>5.17</td>
<td>0.0093</td>
</tr>
<tr>
<td>Strain</td>
<td>1</td>
<td>0.0887</td>
<td>0.0887</td>
<td>6.67</td>
<td>0.0129</td>
</tr>
<tr>
<td>Model × strain</td>
<td>2</td>
<td>0.0051</td>
<td>0.0025</td>
<td>0.10</td>
<td>0.8298</td>
</tr>
<tr>
<td>Temp × model</td>
<td>8</td>
<td>0.0706</td>
<td>0.0088</td>
<td>0.66</td>
<td>0.7196</td>
</tr>
<tr>
<td>Temp × strain</td>
<td>4</td>
<td>0.1506</td>
<td>0.0376</td>
<td>2.83</td>
<td>0.0345</td>
</tr>
<tr>
<td>Model × model × strain</td>
<td>8</td>
<td>0.01617</td>
<td>0.00202</td>
<td>0.15</td>
<td>0.3958</td>
</tr>
</tbody>
</table>

**Fig. 6.** Linear relationship between the growth rates of Rif\(^{+}\) and wild strains of E. coli O157:H7 in MTBM, derived from the modified Gompertz, Huang, and Baranyi models.
was significantly higher than that of Rif<sup>+</sup> strains of E. coli O157:H7 at α = 0.05 level. There was no interaction between the primary model and bacterial strain (P = 0.8298), and between the primary model and temperature (P = 0.7198), suggesting the primary model can be used to fit any growth curves at any temperature condition. However, significant interaction existed (P = 0.0345) between the bacterial strain and temperature, also suggesting that the bacterial strain affected the determination of growth rate. No interaction among temperature, primary, and strain was observed (P = 0.9958, Table 3).

Since the growth rates of Rif<sup>+</sup> and wild strains of E. coli O157:H7 were significantly different, the next natural question was whether there was some kind of correlation between the growth rates of Rif<sup>+</sup> and wild strains of E. coli O157:H7. Fig. 6 demonstrates that there exists a linear relationship (R<sup>2</sup> = 0.92–0.95) between the growth rates of Rif<sup>+</sup> and wild strains of E. coli O157:H7. Depending on the primary model used to fit the growth curves, the growth rate of wild strains of E. coli O157:H7 was 10–20% higher than that of Rif<sup>+</sup> strains of E. coli O157:H7. This observation suggests that it is possible to use the growth rate of Rif<sup>+</sup> strains of E. coli O157:H7 to estimate the growth rate of wild strains of E. coli O157:H7 in MTBM.

3.4. The lag phase

One of the most important hypotheses of the Baranyi model is that <i>h₀</i>, the product of lag phase and growth rate, remains constant. In real world applications, the average of <i>h₀</i> values is used. For the Baranyi model, the average <i>h₀</i> value for Rif<sup>+</sup> and wild-type strains of E. coli O157:H7 was 2.06 ± 0.74 and 2.15 ± 1.14 (mean ± standard deviation, <i>n</i> = 13), respectively. For the modified Gompertz and Huang models, a linear equation (Eq. (10)) was used to correlate the inverse of square-root of lag phase and temperature. Table 4 lists the estimation of β<sub>i</sub> and correlation coefficients for each model and bacterial strains

\[
\frac{1}{\sqrt{L}} = \beta T. \tag{10}
\]

4. Conclusions

This study evaluated and compared the growth kinetics of Rif<sup>+</sup> and wild strains of E. coli O157:H7 in MTBM under selected temperature conditions (5, 10, 15, 20, 25, and 37 °C). It was observed that both types of bacteria did not grow well at temperatures below 10 °C, which agreed well with the nature of mesophilic bacteria. The growth curves of both Rif<sup>+</sup> and wild strains of E. coli O157:H7 in MTBM exhibited sigmoid trends at 15, 20, 25, and 37 °C and were independently modeled by the modified Gompertz, Huang, and Baranyi models, from which the growth rate and lag phase duration at each temperature were determined. Instead of using the traditional Ratkowsky square-root model as a secondary model, it was discovered that the temperature dependence of growth rate was more appropriately described by a modified secondary model, in the form of <i>K</i> = α(T – <i>T<sub>min</sub></i>)<sup>β</sup>. Based on this model, the estimated minimum growth temperature for both Rif<sup>+</sup> and wild strains of E. coli O157:H7 in MTBM was around 6.64–8.76 °C, which is more in agreement with the biological nature of mesophilic bacteria such E. coli O157:H7 than that determined from the traditional Ratkowsky model. Using the traditional Ratkowsky square-root model, the estimated minimum growth temperatures ranged from 1.5 to 4.7 °C, below the real minimum growth temperature for E. coli O157:H7.

While there was no significant difference between the growth rates derived from the modified Gompertz model and Baranyi model, and between those derived from the Huang model and the Baranyi model, the growth rates derived from the modified Gompertz model were significantly higher than those derived from the Huang model at α = 0.05 level. The statistical analysis also suggested that the growth rates were affected by bacterial strains, as the growth rates of Rif<sup>+</sup> E. coli O157:H7 were 10–20% lower than those of wild strains of E. coli O157:H7. It was also interesting to observe that the growth rate of wild strains of E. coli O157:H7 correlated linearly with those of Rif<sup>+</sup> E. coli O157:H7, suggesting that the growth rate derived from Rif<sup>+</sup> E. coli O157:H7 could be used to reliably estimate the growth rates of wild strains of E. coli O157:H7.

For both modified Gompertz and Huang models, the temperature dependence of lag phase of both types of E. coli O157:H7 was described by a linear relationship between the inverse of square-root of lag phase and temperature, with adjusted correlation coefficients (R<sup>2</sup>) ranging from 0.84 to 0.90. For the Baranyi model, the average <i>h₀</i> was 2.06 ± 0.74 and 2.15 ± 1.14 for Rif<sup>+</sup> and wild-type strains of E. coli O157:H7.

In summary, this study provided kinetic analysis pertinent to the growth of E. coli O157:H7 in MTBM. The results of this study can be used to evaluate the microbial safety of E. coli O157:H7 in MTBM during refrigerated storage and under temperature-abused conditions.

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References
