Development and validation of a molecular predictive model to describe the growth of *Listeria monocytogenes* in vacuum-packaged chilled pork

Keping Ye, Huhu Wang, Xinxiao Zhang, Yun Jiang, Xinglian Xu, Guanghong Zhou

Abstract

The aim of this study was to develop a molecular predictive model from appropriate real-time PCR methods, so as to describe the growth of a cocktail of *Listeria monocytogenes* strains in vacuum-packaged chilled pork during storage at selected temperature conditions (4, 10, 15, 20 and 25 °C). We compared this model with a traditional predictive model which used original data obtained by conventional microbiological methods. Real-time PCR was successfully used in the construction of a predictive model. A sigmoidal trend was observed for all growth curves, and four primary growth models (modified Gompertz, Baranyi, Logistic and Huang) could be used to fit the growth curves. The $R^2$ values were >0.97 and MSE values were 0.2198 log cfu/mL in all models used. Most of the $B_f$ and $A_f$ values were within the limit of 1.0 < $B_f$ < $A_f$ < 1.1, except for one obtained by real-time PCR at 25 °C. The $F$ test showed that the modified Gompertz, Logistic and Baranyi models were sufficient to describe growth curves, but the Huang model was rejected twice in ten cases. No difference was observed in accuracy between the molecular and traditional predictive models for most of growth curves when assessed by $F$ test. Further, no differences in both growth rate and lag phase were observed between real-time PCR and conventional microbiological methods. The application of molecular predictive model not only can aid to establish models of certain pathogens more accurately in the presence of other bacteria, but also save time and labor. Thereby, it will reduce the risk of pathogens and enhance the safety of meat and meat products.

1. Introduction

*Listeria monocytogenes* has been recognized as an important food-borne pathogen that causes listeriosis (Anonymous, 2000, p. 143; Mead et al., 1999). This pathogen presents a high mortality rate among some high risk populations. Outbreaks of listeriosis have been associated with raw meat, poultry, milk etc. *L. monocytogenes* is able to grow over a wide range of temperatures (−1.5 to 45 °C), pH values (4.39–9.4), and osmotic pressures (NaCl concentrations up to 10%) (Giffel & Zwietering, 1999). It is also facultatively anaerobic (ICMSF, 1996). Thus early identification of *L. monocytogenes* is a critical issue in public health policy.

Predictive models have been used to study the population dynamics of pathogenic and spoiling bacteria at various times and temperatures during the storage of food. Predictive modeling starts with i) experimental design, ii) data collection and iii) data analysis and summary (as growth rate, lag time, etc) before databases are constructed. With the advancement of predictive modeling and the development of computing (McMeekin, Olley, Ross, & Ratkowsky, 1993; Roberts & Jarvis, 1983), highly sophisticated, detailed models have been developed. For example, modified Gompertz, Logistic, Baranyi and Huang models (Baranyi & Roberts, 1994; Huang, 2004; Zwietering, Jongenburger, Rombouts, & Van’t, 1990) have been described by sigmoid functions. A typical microbial growth curve consists of three phases—first a lag phase, followed by an exponential phase, and finally a stationary phase (Monod, 1949). Meanwhile, to determine whether predictions provide good description of growth in foods, models need to be validated to evaluate their predictive ability. Therefore, predictive mathematical models can be useful in day to day decision making in the food processing operations in HACCP implementation and risk assessment in the event of temperature abuse and process deviation.

Several models for growth of *L. monocytogenes* have been developed (Besse et al., 2006; Crépet, Stahl, & Carlin, 2009; Diez-Gonzalez, Belina, Labuza, & Pal, 2007; Mejhlom et al., 2010; Murphy, Rea, & Harrington, 1996; Pal, Labuza & Diez-Gonzalez, 2008a, 2008b; Sheen, Hwang, & Juneja, 2011). Nevertheless, almost all the original data of predictive models were derived from...
conventional microbiological methods, but the process was time-consuming and labor-intensive. Regarding meat, it can be readily contaminated and is an ideal substrate for various spoilage microorganisms (Jackson, Acuff, & Dickson, 1997; Jay, 2000). Where contaminated and is an ideal substrate for various spoilage consuming and labor-intensive. Regarding meat, it can be readily

2.2. Preparation of samples

Chilled pork samples were purchased from a local retail store. The outer pork layers were removed using a sterile surgical scissor and then discarded. The inner pork samples were divided into 20 g portions (approximately 6 cm x 6 cm x 1 cm) and packaged into sterile plastic bags. Individual samples were then vacuum-packaged using a vacuum-packaging machine (DC800-FBI-E, Promax packaging solution, USA). The bags, containing chilled pork samples, were then treated with high pressure (300 MPa, 10 min at 20 °C) to eradicate all contaminating micro-organisms. The effectiveness of this process for eliminating background bacteria was verified. Following pressure treatment each sample was inoculated with 100 μL of a solution containing approximately 10^6 cfu/mL of L. monocytogenes, and then vacuum-packaged. The initial inoculum of L. monocytogenes in vacuum-packaged chilled pork was approximately 10^3–10^5 cfu/cm².

2.3. Storage and growth measurements

Inoculated vacuum-packaged pork samples were incubated isothermally at 4, 10, 15, 20, and 25 °C during different lengths of time, corresponding to different incubation temperatures. At frequent intervals appropriate for each growth temperature, samples were removed for enumeration of L. monocytogenes. For each temperature studied, quintuplicate growth curves were performed.

2.4. Enumeration of L. monocytogenes

Each vacuum-packaged pork sample (20 g) was homogenized in 80 mL of sterile saline peptone water. After shaking at 230 rpm for 10 min with a stomacher, further steps were undertaken as follows:

2.4.1. Conventional microbiological method

Viable counts were obtained by plating a dilution made in buffered peptone water onto selective agar (PALCAM agar base with selective supplements) (Land Bridge, Beijing, China), and incubated at 37 °C for 72 h. An average cfu/mL of two platings of each growth curve point was recorded and used to determine estimates of the growth kinetics.

2.4.2. SYBR Green real-time PCR assay

A 2-mL aliquot was transferred into a 2 mL sterile tube and centrifuged (Avanti J-E, Beckman Coulter, American) at 200 g (4 °C) for 1 min. The supernatant (1 mL) was aseptically transferred into a 1.5 mL sterile centrifuge tube and a further centrifugation was carried out at 12,000 g for 2 min (4 °C). The pellet was stored at −20 °C until required for extraction of DNA.

Bacterial DNA was extracted using the TiANamp Bacteria DNA Kit (Tiangen Biotech Beijing Co., Ltd., China) according to the manufacturer’s instruction with some modifications. The modifications are mainly prolonging the incubation time of lysozyme (1 h) and proteinase K (2 h), respectively. SYBR Green real-time PCR assay was carried out based on our previous work (Ye et al., 2012), using the primers Hly-f (ACT TCG GCG CAA TCA GTG A) and Hly-r (TTG CAA CTG CTC TTT AGT AAC AGC TT) (Amagliani, Omiccioli, Ribeiro, Bergamini, & Martinis, 2010; Rodríguez-Lázaro, Jofré, Aymeric, Garriga, & Pla, 2004; Rodríguez-Lázaro, Jofré, Aymerich, Hugas, & Pla, 2005; Traunsek et al., 2011; Vanegas, Vásquez, Martinez, & Rueda, 2009). Nowadays, real-time PCR has the potential to be used as a kind of routine methods.

For predictive models, all the original data have been derived from conventional microbiological counting methods. Until now there have been no reports of the development of molecular predictive models from appropriate real-time PCR detection methods. Therefore, the main objective of this study was to develop a molecular predictive model to describe growth of L. monocytogenes in vacuum-packaged chilled pork during storage at selected temperature conditions. Meanwhile, this would allow us to compare molecular predictive model with traditional predictive model obtained by conventional microbiological methods, and also to evaluate the applicability of molecular predictive model for the consequent estimation of the microbial safety of food.

2. Materials and methods

2.1. Bacterial strains used and preparation of inoculum

Five bacterial strains consisting of L. monocytogenes ATCC 19115 (food isolate); L. monocytogenes ATCC 19112 (food isolate); L. monocytogenes ATCC 15313 (food isolate); L. monocytogenes ATCC 19117 (food isolate); L. monocytogenes CICC 21583 (chicken isolate), were used in this study. They were obtained from the China Center of Industrial Culture Collection (Beijing, China).

The five strains of L. monocytogenes were individually maintained frozen at −80 °C in nutrient broth supplemented with 30% glycerol. For the inoculum preparation, frozen suspensions from each strain was thawed and streaked onto selective agar (PALCAM agar base with selective supplements) (Land Bridge, Beijing, China) respectively and the plates were incubated at 37 °C for 48 h. Single colonies from the PALCAM plate were transferred to 10 mL tryptic soy broth (TSB) tubes and were incubated at 37 °C for 4 h and then transferred to another tube of fresh TSB and incubated at 37 °C for 18 h. A cocktail containing all five strains of L. monocytogenes was prepared by mixing 5 mL of each strain (about 10^6 cfu/mL), and it was diluted to about 10^6 cfu/mL as the inoculum.

In recent years, many types of rapid microbial techniques have been developed for quantification of bacteria in food products. One method based on real-time PCR has demonstrated great potential because of its high specificity and sensitivity (Nogva, Rudi, Naterstad, Holck, & Lillehaug, 2000; Rodríguez-Lázaro, Hernandez, et al., 2004). Real-time PCR methods can obtain quantitative terms by construction of standard curves, as well as for the enumeration of L. monocytogenes in food (Bera, Chakraborty, & Maity, 2006; Grady, Sedano-Balbás, Maher, Smithc, & Barryn, 2008; Oliveira, Ribeiro, Bergamini, & Martins, 2010; Rodríguez-Lázaro, Jofré, Aymeric, Garriga, & Pla, 2004; Rodríguez-Lázaro, Jofré, Aymerich, Hugas, & Pla, 2005; Traunsek et al., 2011; Vanegas, Vásquez, Martínez, & Rueda, 2009). Nowadays, real-time PCR has the potential to be used as a kind of routine methods.

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MicroAmp optical eight-tube strips using an ABI Prism 7500 (Applied Biosystems, USA) sequence detection system.

2.5. Mathematical growth modeling of growth of L. monocytogenes on vacuum-packaged chilled pork

Growth curves were generated as the natural logarithm of cfu/mL vs. time using the mean microbial counts at each test time from the five trials at each of the constant temperatures. In Table 1, various models were chosen based on non-linear equations (modified Gompertz, Logistic, Baranyi, and Huang) as growth kinetic models and re-parameterized to reflect microbial growth parameters. Modified Gompertz and Logistic models are empirical, and can sometimes overestimate the maximum growth rates. However, they were widely used in the literature (Diez-González et al., 2007; Giffel & Zwietering, 1999; Huang, 2010; Juneja, Marks, & Thippareddi, 2008; Murphy et al., 1996). Meanwhile, in order to directly compare these models in this study, two more biologically-based growth models were used. The Baranyi model (Baranyi & Roberts, 1994) provided a good fit to the growth data with meaningful microbiological parameters (Juneja et al., 2008). The Huang model (Huang, 2004) used a set of differential equations, representing different stages of bacterial growth, to describe the dynamics of bacterial growth and multiplication.

2.6. Evaluation of model performance

In order to evaluate the goodness-of-fit of the overall models, correlation coefficients ($R^2$), the mean square error (MSE), bias factors (Bf) and accuracy factors (Af) were calculated. Goodness-of-fit of primary model was evaluated using the adjusted $R^2$. The MSE was used to evaluate the difference between the growth data estimated by the model and that measured experimentally; the smaller the MSE is, the better the model will fit. Meanwhile, validation experiments were carried out for evaluating the models by Af and Bf. The Af indicates the spread of the results around the predicted values. The Bf measures the relative average deviation of the predicted and observed bacterial growth. In this study, $0.0 \leq Bf \leq 1.1$ was defined as a satisfactory limit. That is to say, the predictions exceeding observed data and less than 10% on average in terms of log10 (cfu/mL), were considered to be accurate (Yang et al., 2009). $R^2$, RMSE, Af and Bf were defined by the following equations (Baranyi, McClure, Sutherland, & Roberts, 1993; García-Gimeno, Barco, Rincón, & Zurera-Cosano, 2005; Liao, Zhang, Hu, Liao, & Wu, 2008; Ross, 1996; Wang et al., 2007; Zhong et al., 2005; Zurera-Cosano, García-Gimeno, Rodríguez-Pérez, & Hervás-Martínez, 2006).

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(\text{predicted} - \text{observed})^2}{\sum_{i=1}^{n}(\text{observed} - \text{mean})^2}$$  \hspace{1cm} (1)

$$\text{MSE} = \frac{\sum(|\text{predicted} - \text{observed}|)^2}{n-p}$$  \hspace{1cm} (2)

$$\text{Bias factor} = 10 \left( \frac{\sum \log(\text{predicted}/\text{observed})}{n} \right)$$  \hspace{1cm} (3)

$$\text{Accuracy factor} = 10 \left( \frac{\sum \log(\text{predicted}/\text{observed})}{n} \right)$$  \hspace{1cm} (4)

where $n$ is the number of observations, $p$ is the number of model parameters. The observed, predicted and mean are the observed values, predicted values and average values, respectively.

Another way to discriminate among models is to compare them statistically by use of $F$ test (Pal et al., 2008a; Zwietering et al., 1990). The residual sum of squares (RSS, difference between observed and predicted values) was calculated for each growth curve. The Schnute model is a comprehensive model, the RSS of which was taken as an estimate of the measuring error. Therefore, the test models were compared with the Schnute model by testing the $F$-value against the critical $F$-table value showing the 95% confidence level. Where $F$-values were smaller than $F$-values for any growth curve, the model was considered equivalent to a comprehensive model indicating that the test model was sufficient to describe a growth curve. The following was then calculated:

Table 1 Mathematical growth models used to predict growth of L. monocytogenes.

<table>
<thead>
<tr>
<th>Model Type</th>
<th>Equation</th>
<th>Parameter$^a$</th>
</tr>
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<tbody>
<tr>
<td>Modified Gompertz$^a$</td>
<td>$Y = Y_0 + (Y_{max} - Y_0)\exp{-\exp[-\mu^*(t - M)]}$</td>
<td>$\lambda = \frac{M - 1}{\mu}$, $K = \frac{B - A^*\mu}{\mu}$</td>
</tr>
<tr>
<td>Logistic$^a$</td>
<td>$Y = Y_0 + \frac{(Y_{max} - Y_0)}{1 + \exp[-\mu^*(t - M)]}$</td>
<td>$\lambda = \frac{M}{2}$, $K = \frac{B - A^*\mu}{\mu}$</td>
</tr>
<tr>
<td>Baranyi$^b$</td>
<td>$Y = Y_0 + \lambda^e(t + \ln(\exp(-K^*t) + \exp(-h_0) + \exp(-K^*t - h_0))$</td>
<td>$\lambda = \frac{\lambda}{K}$</td>
</tr>
<tr>
<td>Huang$^c$</td>
<td>$Y = Y_0 + Y_{max} - \ln(\exp(Y_0)) +</td>
<td>\exp(Y_{max}) - \exp(Y_0)</td>
</tr>
<tr>
<td>Schnute$^d$</td>
<td>$Y = \left(\frac{\mu^<em>(1 - b/a)^{\lambda}}{1 - b^</em>\exp(\mu^<em>(t - M)) + 1 - b - a</em>\lambda}\right)$</td>
<td>$-\lambda$</td>
</tr>
</tbody>
</table>

$^a$ As derived by Zwietering et al. (1990).
$^b$ As derived by Baranyi and Roberts (1994).
$^c$ As derived by Huang (2004).
$^d$ As derived by Pal et al. (2008a).
$^e$ $Y$: the natural logarithm of bacteria count at any given time, ln cfu/mL; $Y_{max}$ and $Y_0$ are the maximum and initial values of $Y$; $\mu$: the relative growth rate growth rate at time $- M$, which is the inflection point of the curve; $t$: lag phase; $K$: specific growth rate; $a$, $b$: model coefficients.
If $DF_{\text{test}} \neq DF_{\text{Schnute}}$ then $f = \frac{(RSS_{\text{test}} - RSS_{\text{Schnute}})/(DF_{\text{test}} - DF_{\text{Schnute}})}{RSS_{\text{Schnute}}/DF_{\text{Schnute}}}$ tested against $F_{DF_{\text{test}} - DF_{\text{Schnute}}}$.

If $DF_{\text{test}} = DF_{\text{Schnute}}$ then $f = RSS_{\text{test}}/RSS_{\text{Schnute}}$ against $F_{DF_{\text{test}}}$.

Graphical comparison was used by plotting values for growth rate or lag phase from conventional microbiological methods against the corresponding growth rate ($K$) or lag phase ($\lambda$) obtained by appropriate real-time PCR detection methods. From this plot, we can obtain the difference between the models derived from two detection methods. For this, examination and analysis of residual plots can also be useful.

2.7. Statistical analysis

The $L. \text{monocytogenes}$ growth data obtained at isothermal conditions were fitted to the models using Marquardt iterative method employing a non-linear regression procedure, PROC NLIN, in SAS package (Release 8.1, SAS Institute Inc., Cary, NC). Defining the starting values in the SAS program to estimate the parameters is very critical as inappropriate values, if used, cannot be converged and can lead to over or under estimation of the parameters. The given starting values, after several iterations in the nonlinear procedure, converged to estimated values of the parameters (Velugoti et al., 2011).

Residual analysis was used by SPSS 18.0 to compare the difference in the growth rate ($K$) or the lag phase ($\lambda$) derived from conventional microbiological and real-time PCR method.

3. Results and discussion

In recent years, a large number of predictive mathematical models have been developed to predict the growth of pathogenic organisms during the storage of meat and meat products (Gunvig, Hansen, & Borggaard, 2013; Juneja, Marks, Huang, & Thippareddi, 2011; Kajak & Kotožyn-Krajewska, 2006; McDonald & Sun, 1999; Mejholm et al, 2010; Pal et al., 2008a; Yang et al., 2009). However, these traditional predictive models are time-consuming and can lead to deviations in accuracy in the presence of background bacteria (Brul, Gerwen, & Zwietering, 2007; Huang, 2010). In this study we used treated vacuum-packaged chilled pork inoculated with $L. \text{monocytogenes}$ as a model, so as to eliminate counting interference of other micro-organisms, and contribute to obtain the accurate counting by conventional microbiological counting method. Simultaneously, physiological and molecular information will be increasingly available for incorporation into models (McMeekin et al., 2008). Therefore, we attempted to develop molecular predictive models based on the original data of experiments by means of appropriate specific real-time PCR detection methods. Different models, such as modified Gompertz, Logistic, Baranyi and Huang models were used for fitting, and they were each evaluated for their predictive ability and feasibility. As well, we compare the molecular predictive model with traditional predictive model.

3.1. Real-time PCR used in predictive models

In predictive microbiology, almost all the models were obtained by the original data derived from selective enumeration, some researches reported that selective medium was not very selective, and always needed to add some antibiotics to improve its specificity (Darukaradhya, Phillips, & Kailasapathy, 2006; Robert-Pillot, Baron, Lesne, Fournier, & Quilici, 2002; Talwalkar & Kailasapathy, 2004). However, for the antibiotic-resistant bacteria, they didn't represent most of the same kind bacteria (Huang, 2010; Oberg, Moyes, Domek, Brothersen, & McMahon, 2011). Simultaneously, the process of conventional microbiological method was time-consuming and labor-intensive. Because of its high specificity and accuracy, real-time PCR has been widely used to detect a number of kinds of bacteria in meat and meat products (Alonso, Herrero, Vieites, & Espiñeira, 2011; Brightwell & Clemens, 2012; Delibato et al., 2010, p. 131; McGuinness et al., 2009). Therefore, in this study, we used SYBR Green real-time PCR assay for obtaining the origin data of the predictive model, which was based on our previous work (Ye et al., 2012). In our previous work, we found the DNA-based quantitative real-time PCR assay was more accurate than RNA-based assay when tested samples were untreated. Simultaneously, Meat and meat products have various background spoilage bacterial flora, some of which can also grow in certain selective medium, that will affect the plate counting of selected bacteria. However, real-time PCR uses specific primers by which $L. \text{monocytogenes}$ can be distinguished from the remaining background bacterial flora. The specificity test of this method was assessed with the $L. \text{monocytogenes}$ and a collection of 41 other bacteria commonly found in meat, and this method has high specificity. Therefore, by the construction of standard curves in quantitative detection of SYBR Green DNA-based real-time PCR, we obtained colony forming units of $L. \text{monocytogenes}$ during the storage of vacuum-packaged chilled pork.

3.2. Preliminary fitting and observation of isothermal growth curves of both molecular and traditional predictive models

The growth curves of a cocktail of five $L. \text{monocytogenes}$ strains on vacuum-packaged chilled pork at different constant temperatures (4, 10, 15, 20 and 25 °C) were constructed. Fig. 1 shows the growth curve of $L. \text{monocytogenes}$ in vacuum-packaged chilled pork at constant temperatures obtained by conventional microbiological methods. Fig. 2 shows the growth curve of $L. \text{monocytogenes}$ in vacuum-packaged chilled pork at constant temperature obtained by means of real-time PCR. $L. \text{monocytogenes}$ grew well from 4 °C to 25 °C. Four primary growth models (modified Gompertz, Logistic, Baranyi and Huang models) were used for fitting (see Figs. 1 and 2). Modified Gompertz and Logistic models can represent most of empirical models, which have been wildly used in the literature, however they always overestimate the maximum growth rate (Huang, 2010; Rosso, Lobry, Bajard, & Flandrois, 1995). Therefore, they were used as reference models. To directly compare these models, two other more biologically based growth models called Baranyi and Huang models were used in this study (Buchanan, Whiting, & Damert, 1997). From Figs. 1 and 2, we found that all the isothermal growth curves were sigmoidal, and exhibited lag, exponential, and stationary phases. That is to say, all four models, modified Gompertz, Logistic, Baranyi and Huang models, were suitable for describing the growth of $L. \text{monocytogenes}$ inoculated into vacuum-packaged chilled pork samples when incubated at 4, 10, 15, 20 and 25 °C. Both molecular and traditional predictive growth curves could be fitted by the four models. Each data fitting figure showed that there were similar numbers of colony forming units of $L. \text{monocytogenes}$ at the stationary phase using each of the four different models, but there was a small deviation of the colony
forming units with the initial bacterial value. Compared with the modified Gompertz, Logistic and Baranyi models, the Huang model had a more clearly identifiable lag phase as observed by Huang (2010). The Logistic model and the Baranyi model resembled each other in the lag phase, whereas with the modified Gompertz model, the period as the exponential phase turned into the stationary phase, the values were lower compared with the other models.

3.3. Estimation of parameters and comparison of growth curves fitted by different models

All the growth curves containing molecular and traditional predictive models were evaluated by correlation coefficient ($R^2$), the mean square error (MSE), bias factor (Bf) and accuracy factor (Af) values. Parameter values and performance statistics of four fitted equations at each measured temperature are shown in Table 2. The resulting $R^2$ values were >0.97 and MSE values were 0.2198 log cfu/mL in each of the four models, and thus all met the defined requirements (Lopez, Prieto, Dijkstra, Dhanoa, & France, 2004; Ross, 1996). It can be seen that the modified Gompertz model provided a good statistical fit to the observed data and its $R^2$ values were closest to 1 in all four models; however, the differences were often small. The estimated MSE values were within the precision of microbial enumeration, indicating that four different models fitted isothermal growth data well. The small MSE value suggested that the model was reasonably accurate in describing the effect of time on the colony forming units of L. monocytogenes at constant temperature (Giffel & Zwietering, 1999). Therefore, the fitting results of all the models were available.

Additional five data sets of each temperature were determined to compare observed values with model predictive values, and the bias factor (Bf) and accuracy factor (Af) values were calculated by
Eqs. (3) and (4) to assess the performance of the developed models. From Table 2, it can be observed that almost all of the $B_f$ and $A_f$ values of the models were within the limit of $1.0 \leq B_f \leq A_f \leq 1.1$, except for the models obtained by real-time PCR at $25 \, ^\circ C$. The indices bias and accuracy provide an objective indication of model performance. A bias factor less than 1 indicates that a model is, in general, fail-safe (Pal et al., 2008b). However, the bias factor provides no indication of average accuracy of estimates because under- and over-predictions tend to cancel out (Yang et al., 2009). Therefore, the accuracy factor should be calculated. As shown in Table 2, the average $A_f$ of models obtained by conventional microbiological method was 1.0375, and that by real-time PCR method was 1.039. The results suggested that the predictions were almost identical with observations in all the models, and the predicted curves could accurately describe the growth of *L. monocytogenes* on vacuum-packaged chilled pork at constant temperature.

The performance of the four models (modified Gompertz, Logistic, Baranyi and Huang model) was also evaluated by $F$-test. The premise of the $F$-test was that the Schnute model is a comprehensive model and closely predicts the microbial counts, and the RSS of the Schnute model was considered as an estimate of the measuring error (Pal et al., 2008a). Many researches used $F$-test method to assess the statistical significance of the difference between models in terms of the goodness-of-fit to the same set of data, and the statistical performance could lead to some suggestions for the evaluation of models (Lopez et al., 2004; Motulsky & Ransnas, 1987; Pal et al., 2008a; Zwietering et al., 1990). In Fig. 3, the $f$-values are plotted against the $F$-values for the growth of *L. monocytogenes* strains on

![Fig. 2. Growth curves of *L. monocytogenes* on vacuum-packaged chilled pork at constant temperature (4, 10, 15, 20 and 25 °C) obtained using real-time PCR detection method. The empty squares represent the mean values of quintuplicate trials of raw data points; the blue lines represent the modified Gompertz model; the red lines represent the Logistic model; the yellow lines represent the Baranyi model; the green lines represent the Huang model. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)](image)
vacuum-packaged chilled pork at 4, 10, 15, 20 and 25 °C, and the results from the F-test for the modified Gompertz, Logistic, Baranyi and Huang models are shown. If the f-testing value was smaller than the F table value, the model was accepted. The f-values of modified Gompertz, Logistic and Baranyi models were all smaller than the critical F-table values (95% confidence), and so the three models were sufficient to describe growth curves, and the differences between the three models were very small. However, it can be seen that only the Huang model was rejected by these criteria, i.e., twice in ten cases. This study also showed that no significant difference of accuracy existed between molecular and traditional predictive models for the most of growth curves.

### 3.4. Effect of real-time PCR detection method on determination of construction of models by comparison of specific growth rates and lag phase durations

When vacuum-packaged chilled pork inoculated with \textit{L. monocytogenes} was incubated at constant temperatures from 4 °C to 25 °C, \textit{L. monocytogenes} grew as expected. The growth rate

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**Table 2**

Evaluation of general models (modified Gompertz, Logistic, Baranyi and Huang model) predicting the growth of \textit{L. monocytogenes} on vacuum-packaged chilled pork.

<table>
<thead>
<tr>
<th>T</th>
<th>Model 1&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Model 2&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gompertz</td>
<td>Baranyi</td>
</tr>
<tr>
<td>4 °C</td>
<td>( R^2 )</td>
<td>0.9994</td>
</tr>
<tr>
<td></td>
<td>MSE</td>
<td>0.0326</td>
</tr>
<tr>
<td></td>
<td>( B_f )</td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td>( A_f )</td>
<td>1.05</td>
</tr>
<tr>
<td>10 °C</td>
<td>( R^2 )</td>
<td>0.9998</td>
</tr>
<tr>
<td></td>
<td>MSE</td>
<td>0.0107</td>
</tr>
<tr>
<td></td>
<td>( B_f )</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>( A_f )</td>
<td>1.04</td>
</tr>
<tr>
<td>15 °C</td>
<td>( R^2 )</td>
<td>0.9995</td>
</tr>
<tr>
<td></td>
<td>MSE</td>
<td>0.0211</td>
</tr>
<tr>
<td></td>
<td>( B_f )</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td>( A_f )</td>
<td>1.01</td>
</tr>
<tr>
<td>20 °C</td>
<td>( R^2 )</td>
<td>0.9993</td>
</tr>
<tr>
<td></td>
<td>MSE</td>
<td>0.0198</td>
</tr>
<tr>
<td></td>
<td>( B_f )</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td>( A_f )</td>
<td>1.05</td>
</tr>
<tr>
<td>25 °C</td>
<td>( R^2 )</td>
<td>0.9995</td>
</tr>
<tr>
<td></td>
<td>MSE</td>
<td>0.0398</td>
</tr>
<tr>
<td></td>
<td>( B_f )</td>
<td>1.03</td>
</tr>
<tr>
<td></td>
<td>( A_f )</td>
<td>1.03</td>
</tr>
</tbody>
</table>

<sup>a</sup> The original data of these parameters were obtained by conventional microbiological method.

<sup>b</sup> The original data of these parameters were obtained by means of appropriate real-time PCR method.

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Fig. 3. F-test on \textit{L. monocytogenes} growth data as fitted by modified Gompertz, Logistic, Baranyi and Huang models at different constant temperatures. The f value was derived from a conventional microbiological method; the f value was derived from a real-time PCR method.
(log cfu/mL/h) increased with temperatures and varied between 0.03 and 0.5 log cfu/mL/h, and the lag time (h) decreased from 42 to 3 h. Temperature is a major and reproducible factor determining the growth of *L. monocytogenes*, with the rate increasing with increased storage temperature. Lag phases were apparent in all models, and those samples stored at low temperatures had longer lag phases than those at high temperatures. The growth rate and lag time calculated by traditional predictive model were consistent with the published ones which were listed by Augustin & Carlier, 2000. Therefore, the rates derived from two detection methods were compared for growth of *L. monocytogenes*. As can be observed in Fig. 4A, most points fell close to the line of equivalence, that is to say, the growth rate value obtained by real-time PCR was almost equal to that by conventional microbiological methods. In Fig. 4B the comparison of the lag phase derived from two detection methods in vacuum-packaged chilled pork was given for all the models. Like the growth rate, no difference between the lag phase derived from real-time PCR nor conventional microbiological methods could be noted. Plots of residuals between the lag phase derived from real-time PCR nor conventional microbiological methods could be noted. Plots of residuals between the lag phase derived from real-time PCR and conventional microbiological methods. In Fig. 4A the comparison of the lag phase derived from real-time PCR was almost equal to that by conventional microbiological methods. In Fig. 4B the comparison of the lag phase derived from real-time PCR and conventional microbiological methods.

### 4. Conclusion

This study evaluated and compared molecular predictive models with the traditional predictive model for the growth of *L. monocytogenes* in vacuum-packaged chilled pork under selected temperature conditions (4, 10, 15, 20 and 25 °C). Pooling over all experiments, the molecular predictive model was similar to the traditional predictive model; that is the molecular predictive model used to predict the development of *L. monocytogenes*. In addition, the modified Gompertz, Logistic, Baranyi and Huang models could be fitted for this type of molecular predictive model. Further research is required to apply and validate this model for the construction of models in other meat and meat products with various types of bacteria are present. The molecular predictive model used here can aid to establish growth patterns of certain pathogens more accurately and thus reduce the risk of pathogens to ensure the safety of meat and meat products.

### Acknowledgments

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**Fig. 4.** Comparison of growth parameters (μ and μ', λ and λ') obtained from conventional microbiological and real-time PCR detection method in all the models. μ (log cfu/h) and λ (h): derived from the front method; μ' and λ': derived from the latter method.

**References**


