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Research Article

Predictive Model for Growth of *Listeria monocytogenes* on the Surface of Fresh Beef

[1]Chen Xiangbo, [2]Ye Sudan, [1]Xiong Xiong, [1]Ye Mengfei and [1]Chen Chun
[1]Zhejiang Provincial Key Laboratory of Biometrology and Inspection and Quarantine Technique, College of Life Sciences, China Jiliang University,
[2]Zhejiang Economic and Trade Polytechnic, Hangzhou 310018, P.R. China

Abstract: This study was sought to establish a predictive model for *Listeria monocytogenes* growth on the surface of fresh beef. Fresh beef were totally disinfected with ozone water prior to inoculate with *L. monocytogenes* and box-packaged to store at 5, 10, 15, 20, 25 and 30°C to calculate the CFU to predict the growth trend. The results showed that the growth trend of *L. monocytogenes* on fresh beef surface could be well fitted with modified Gompertz models (R^2>0.98). Temperature effect on maximum specific growth rate and lag phase was demonstrated by the square-root model as a good linear relationship with R^2 values of 0.93 and 0.87, respectively. Conclusively, a predictive growth model for *L. monocytogenes* on surface of fresh beef stored at various temperature from 5 to 30°C was established effectively as follows: $LgN_t = lgN_0+lg(N_{max}/N_0)\times Exp\{-Exp[2.718\times(-0.002137+0.002203t) 2/lg(N_{max}/N_0)\times((0.004544+0.015048t)^{-2}-t)+1]\}$.

Keywords: Fresh beef, *Listeria monocytogenes*, prediction model

INTRODUCTION

Predictive food microbiology was a new discipline based on microbiology, mathematics, statistics and computer science (Li *et al*., 2001; Xu, 1995; Ross, 1996; Liu *et al*., 2007). It can use mathematical model to quantitative analyze the growth and survival trends of food pathogens or spoilage bacteria under different environmental conditions, make a reasonable forecast for possible risk. Therefore, it is significant to establish dynamic growth models of the microorganism which related to the food quality and can describe microbial behavior under various temperature conditions (Dalgaard, 1995).

Listeria monocytogenes, one of zoonotic foodborne patheogen (Ding *et al*., 2010), has a low incidence but high death rate of 20%-30%. People who are pregnant, infants, the elderly or immunocompromised will get higher risk of infection. Furthermore, *L. monocytogenes* is also one of the main pathogens in frozen food since it can still keep survival and proliferation at low temperature. Inadequate cold chain system which enables to accelerate the potential detrimental pollution due to a rapid proliferation of *L. monocytogenes* during transmitting process will threaten the quality and safety of beef production. Therefore, to develop a predictive model for forecasting the microbial growth is helpful to ensure the quality and safety of food. However, the established predictive models are usually based on data derived from the meat material that washed with sterile water or sterilized at high temperature and high pressure (Zhu, 2008; Armitage, 1997; Mcmeekin *et al*., 2008), which can not depict the real microbiogical growth on crude materials. This study was to establish a mathematical model that accurately predicted the growth of *L. monocytogenes* on the surface of crude fresh beef under different temperature conditions and provide the useful suggestions for risk assessment of *L. monocytogenes* in the beef.

MATERIALS AND METHODS

Fresh samples preparation and pretreatment: The fresh beef obtained from Zhejiang Suichang beef processing company, were packaged into sterile bags and directly frozen in -20°C. Prior to inoculation with *L. monocytogenes*, the fresh beef have been shaped into 1 cm-diameter pieces (approx 1 g weight). In order to eradicate the possible microbe existing on the surface, the fresh samples have been disinfected with 10 mg/L ozone water for 5 min under the sterile operation environment (Qiu and Fang, 2007). This disinfection method has been evaluated for reliability by the daily sterile observation on the bacterial culture medium.

Test organisms preparation: *L. monocytogenes* ATCC19114-3 was inoculated in tryptic soy agar-yeast extract (TSB-YE) liquid medium, shocked at 37°C

Corresponding Author: Chen Chun, Zhejiang Provincial Key Laboratory of Biometrology and Inspection and Quarantine Technique, College of Life Sciences, China Jiliang University, Xueyuan Street No. 258, Xiasha High Education Area, Hangzhou 310018, Zhejiang Province, P.R. China

overnight. Concentration was adjusted to 10^3 cfu/mL for subsequent study.

Temperature variation studies: 0.1 ml *L. monocytogenes* liquid culture with 10^3 cfu/mL ultimate concentration was added on the surface of beef samples, then they were incubated in 2×2 cm sterile medicine box for subsequent growth observation with various temperature treatments described as follows. Treatments under 5°C incubated for 28 d were counted with CFU every 2 d. Treatments under 10°C incubated for 14 d were counted with CFU every 24 h, Treatments under 15°C incubated for 156 h were counted with CFU every 12 h, Treatments under 20°C incubated for 88 h were counted with CFU every 8 h; Treatments under 25°C incubated for 40 h were counted with CFU every 4 h; Treatments under 30°C were counted with CFU by 2, 4, 6, 8, 10, 15, 20, 25, 30 and 35 h, respectively. All tests were repeated three times. All samples were washed by 3 mL physiological saline then the liquid residues were incubated on the Brian Heart Infusion (BHI) agarose for CFU-counting with the Chinese national standards (GB 47892, 2010).

Predictive model development:

- **Growth predictive model of colonies number as primary growth model:** Gompertz model can well describe the growth trend of microorganism. In this study, a modified Gompertz equation was developed to describe the growth of *L. monocytogenes* under various temperatures. The equation is following:

$$LgN_t = lgN_0+lg(N_{max}/N_0)\times Exp\{-Exp[2.718\times\mu_{max}/lg(N_{max}/N_0)\times(\lambda-t)+1]\} \quad (1)$$

where,

N_t = The number of viable microorganisms at various temperature
N_0 = The initial microbial number
N_{max} = The maximum microbial number in stable phase
μ_{max} = The maximum specific growth rate of microbe
λ = Microbial lag phase time

- **Square root model as secondary growth model:** The square root equation, presented by Ratkowsky *et al*. (1982), Buchanan *et al*. (1997) and Baranyi and Roberts (1994) as an experience model which could describe the linear relationship of microorganisms under different temperature conditions between growth rate or inverse square root of lag phase and effective temperature, could well illustrate the growth of microorganisms under different temperature conditions. The formula is as follows:

$$\sqrt{\mu_{max}} = b_\mu \times (T - T_{min\ \mu}) \quad (2)$$

$$\sqrt{1/\lambda} = b_\lambda \times (T - T_{min\ \lambda}) \quad (3)$$

In the Eq. (2) and (3), where T is the Celsius temperature (°C) and T_{min} is a hypothetical value, represented the temperature when specific growth rate is zero, parameter b is the constant value.

- **Statistics analysis:** All analysis and statistics in this study were performed by DPS software (Tang and Feng, 2002).

RESULTS AND DISCUSSION

Growth curve of *L. monocytogenes* under various temperatures condition: Growth curves of *L. monocytogenes* under various temperatures were illustrated in Fig. 1. A, B, C, D represented the growth curve of *L. monocytogenes* at 30, 25, 20 and 15°C, respectively. These curves showed as a steep rise in the

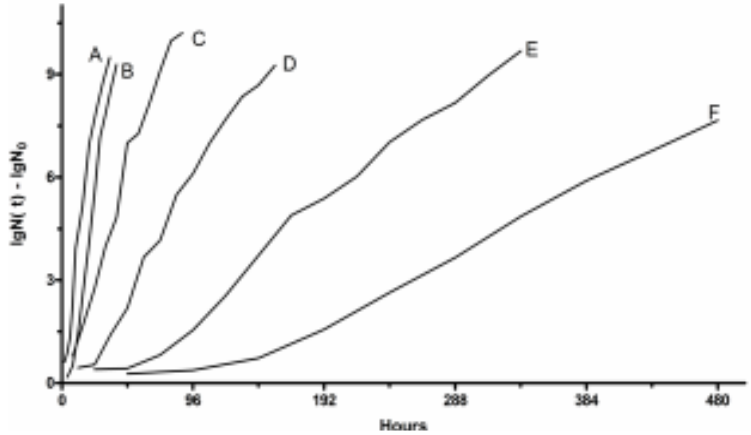

Fig. 1: Growth curve of *L. monocytogenes* at various temperatures. A, B, C, D, E and F represent the growth curve of *L. monocytogenes* at 30°C, 25°C, 20°C, 15°C, 10°C and 5°C, respectively

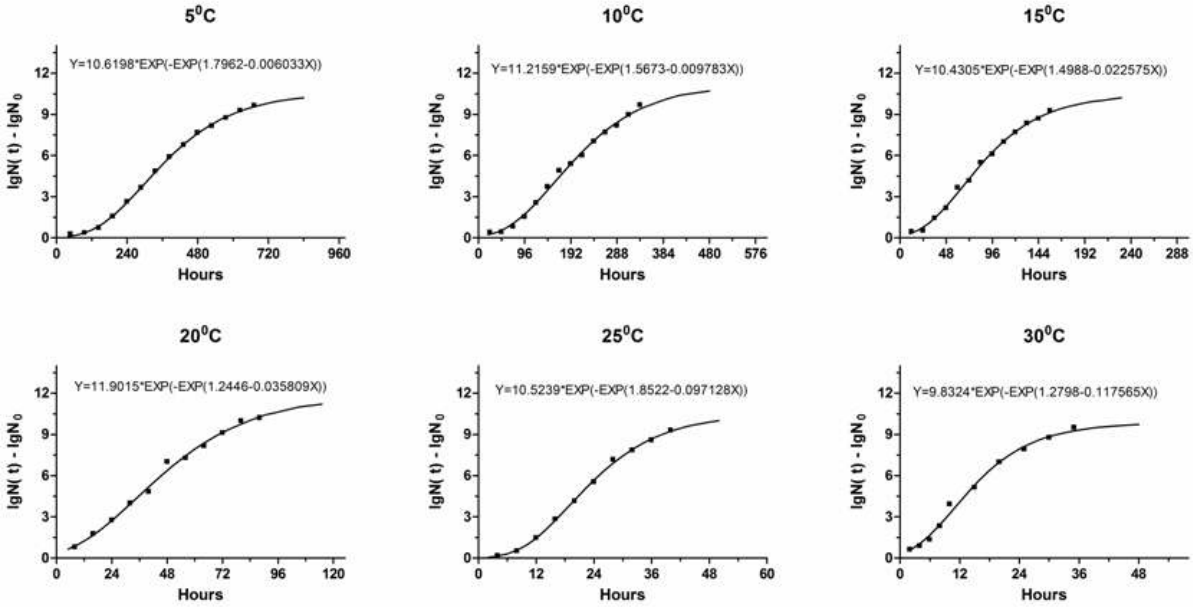

Fig. 2: Growth tendency of *L. monocytogenes* on the surface of fresh beef at various temperatures (Solid dots: observation values, solid line: fitted curve). The equation shows the estimated parameter of modified Gompertz model under relevant temperatures. All parameters are highly significant ($p<0.01$) and the correlation coefficient (R^2) is more than 0.98

first 96 h culture period obviously suggested that *L. monocytogenes* could rapidly grow at the range of 15 to 35°C and the concentration may reach the risk threshold of food safety during those culture periods. The upward trend of the curves E and F (the growth curve of *L. monocytogenes* at 10 and 5°C) were not showed up obviously but began to rise rapidly after 96 h. It showed that *L. monocytogenes* could also cause huge risks in low temperature condition as the time extended.

Primary growth model of *L. monocytogenes*: Figure 2 showed that the modified Gompertz equation can better predict the dynamic growth of *L. monocytogenes* at 30, 25, 20, 15, 10 and 5°C, respectively. Gompertz model was used to fit the experimental data by the optimized method of Marquardt. Additionally, Kinetic parameters in Table 1 demonstrated that maximum number in stable phase (N_{max}) of *L. monocytogenes* was 11.9015±0.6322 at the condition of 20°C and the minimum value was 9.8324±0.4249 at the condition of 30°C. The maximum specific growth rate (μ_{max}) increased with the temperature increasing. The maximum value of μ_{max} was 0.004399153/h at the condition of 30°C, the minimum value was 0.00020901/h at the condition of 5°C as well. The time of lag phase (λ) was decreasing as the temperature increasing. The maximum value was 131.974 at 5°C; the minimum value was 2.3799 h occurred at 30/°C.

Development and verification of secondary growth model: As the limits of primary model (demonstrated as Gompertz model above), square root equation was performed to describe the influence of different temperature for the maximum specific growth rate (μ_{max}) and the time of lag phase (λ). Fitness of the curve of maximum specific growth rate-temperature pair ($\sqrt{\mu_{mzx}} - t$) and lag phase-temperature pair ($\sqrt{1/\lambda} - t$) were calculated by DPS statistical software. As results, the equation of relationship between temperature and maximum growth rate and lag phase are following:

Table 1: Kinetic parameters of *L. monocytogenes* grown on the surface of fresh beef at various temperatures based on modified Gompertz model

T/°C	N_{max}[lg(CFU)]	μ_{max}/h	λ/h	SSE	R^2
5	10.6198±0.1313	0.000209010	131.974	0.0071	0.9995
10	11.2159±0.5605	0.000320914	57.9883	0.0576	0.9954
15	10.4305±0.3402	0.000796294	22.0952	0.0333	0.9972
20	11.9015±0.6322	0.001106983	6.83070	0.0830	0.9940
25	10.5239±0.2660	0.003395614	8.77400	0.0162	0.9989
30	9.8324±0.42490	0.004399153	2.37990	0.1052	0.9929

$$\sqrt{\mu_{max}} = -0.002137+0.002203T \quad (4)$$

$$\sqrt{1/\lambda} = 0.004544+0.015048T \quad (5)$$

In those equations, correlation coefficients (R^2) are 0.93 and 0.87, respectively. b = 0.002203 and T_{min} = 0.97041 can be calculated according to the Eq. (4). So we infer that *L. monocytogenes* could grow at 0.97°C theoretically. By merging Eq. (4) and (5) into Eq. (1), the growth kinetics model of *L. monocytogenes* at 5-30°C in fresh beef could be acquired. The equation is as follow:

$$LgN_t = lgN_0+lg(N_{max}/N_0)\times Exp\{-Exp[2.718\times$$

$(-0.002137+0.002203t)^2/\lg(N_{max}/N_0)\times$
$((0.004544+0.015048t)^{-2}-t)+1]\}$

CONCLUSION

Conclusively, the primary growth model of Gompertz equation could well describe the growth of *L. monocytogenes* under various temperatures on the surface of fresh beef and secondary model of square root could fit well the bacteria growth rate and the time of lag phase. In addition, the results showed that *L. monocytogenes* could grow at 0.97°C which consistent with one of their biological traits (could grow under low-temperature) and this could improve their health hazard being a food borne pathogen. Therefore, our experimental data have embodied the general trend of the bacterial growth and can predict the growth of *L. monocytogenes* under various temperatures, enrich the database of *L. monocytogenes* proliferation in various meats. However, this study only developed the predictive model about the growth of *L. monocytogenes* at multiple constant temperatures but not at the variable temperature, so the influence of temperature fluctuation during transportation on their growth in beef still need further research.

REFERENCES

Armitage, N.H., 1997. Use of predictive microbiology in meat hygiene regulatory activity. Int. J. Food Microbiol., 36: 103-109.

Baranyi, J. and T.A. Roberts, 1994. A dynamic approach to predicting bacterial growth in food. Int. J. Food Microbiol., 23(3/4): 277-294.

Buchanan, R.L., R.C. Whiting and W.C. Damert, 1997. When is simple good enough: A comparison of the Gompertz, Baranyi and three-phase linear models for fitting bacterial growth curves. Food Microbiol., 14: 313-326.

Dalgaard, P., 1995. Modelling of microbial activity and prediction of shelf life for packed fresh fish. Int. J. Food Microbiol., 26(3): 305-317.

Ding, T., Q.L. Dong, L. Wang, M.H. Ma and Y.G. Jin, 2010. Establishment and validation of predictive model for *Listeria monocytogenes* in broth. J. Huazhong Agric. Univ., 29(4): 522-526.

GB 47892, 2010. Food microbiological examination: Aerobic plate count. National Food Safety Standard.

Li, B., L.T. Li and E. Tatsumi, 2001. Development of predictive microbiology. Food Fermentation Ind., 27(11): 54-57.

Liu, W., W.D. Liu and H.Q. Ying, 2007. Predictive microbiology and the application in the field of food safety. Chin. Preventive Med., 8(4): 511-512.

Mcmeekin, T.A., J. Bowman and O. Mcquestin, 2008. The future of predictive microbiology: Strategic research, innovative applications and great expectations. Int. J. Food Microbiol., 128(1): 2-9.

Qiu, J. and Y.M. Fang, 2007. Evaluation of germicidal efficacy of ozone water by two methods. Chin. J. Disinfect., 24(6): 515-517.

Ratkowsky, D.A., J. Olley, T.A. McKeekin and A. Ball, 1982. Relationship between temperature and growth rate of bacterial cultures. J. Bacteriol., 149: 1-5.

Ross, T., 1996. Indices for performance evaluation of predictive models in food microbiology. J. Appl. Bacteriol., 81: 501-508.

Tang, Q.Y. and M.G. Feng, 2002. DPS Data Processing System for Practical Statistics. Science Press, Beijing.

Xu, T.Y., 1995. The forecast model of food microorganism growth. Food Sci., 16(1): 17-21.

Zhu, T.M., 2008. Dulplex PCR Identification and Molecular Typing of *Listeria monocytogenes* in Frozen Products. Huazhong Agricultural University Library, Wuhan.