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Validation of Predictive Liquid Model Systems for the Growth of *Listeria monocytogenes* and *Yersinia enterocolitica* on Pork at Various Temperatures

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Abstract The present study was carried out to envisage the aerobic growth of *Listeria monocytogenes* and *Yersinia enterocolitica* on pork, which is one of the major meat sources in Korea. The results were compared with the previously developed predictive model systems for the verification of microbial growth in a real situation during pork processing. Pork loin samples (8.0 g, 5 mm thick) were aseptically prepared and inoculated with each pathogen by immersing into the respective inoculums for one min. Each of the samples were then wrapped with PE film and stored at 5, 10, and 15°C up to 36 days to measure the growth profile of the respective pathogens. The growth parameters were calculated by using Gompertz equation and were compared with the previously reported data. The predicted generation time (GT) of *L. monocytogenes* at 5, 10 and 15°C was 28.74, 7.85 and 4.02 hr, respectively, and for *Y. enterocolitica* was 10.29, 4.74 and 2.50 hr, at the same temperatures respectively. In this study, the GT values predicted on pork were slightly higher than the values predicted in other studies using liquid model systems. Unlike previous reports, both the pathogens were found to grow at 5°C on pork. This finding recommends the necessity of controlling the growth of both the pathogens during the slaughtering process and distribution.

Keywords: predicting, growth, *L. monocytogenes*, *Y. enterocolitica*, pork

Introduction

Due to the increase in foodborne outbreaks in terms of frequency and size, the interest and need on food safety is enormously increasing all over the world. Especially, meat and meat products are the major source of microbial contamination, and hence are the leading cause of foodborne illness. In Korea, there have been 14 outbreaks due to microbial contamination of meat and meat products in 2003, and they were considered as the top 3 leading causes of foodborne illness along with ready-to-eat food and fishery products (1).

Though the nature of food is same in day-to-day life, the number of microorganisms inhabiting in a food item varies according to the environmental and storage conditions of the food. Therefore, the concept of predictive microbiology is necessary to control the microbial hazard of food in various processing conditions. The main interest of predictive microbiology is that the growth and death of pathogenic microorganisms are described in mathematical terms in a specific condition to predict the microbial hazard and to control the safety of food (2).

Many studies on predictive microbiology have been conducted on *Staphylococcus aureus* (3, 4), *Escherichia coli* O157:H7 (5, 6), *Salmonella* (7), *Yersinia enterocolitica* (8, 9), *Listeria monocytogenes* (10-12), and *Clostridium perfringens* (13), and several mathematical models have been developed including software programs such as, USDA Pathogen Modelling Program and Food Micro-Model. Lee *et al.* (14) and Rho *et al.* (15) have utilized the aforementioned models to predict the maximum edible time of *Kimbab* and the contamination level of pathogens in pork processing, but the studies have remained incomplete (16). Hence, there arises a need to evaluate/apply the mathematical models in studying the real food systems. (17).

In Korea, the consumption of meat and meat products including pork has been constantly increasing year wise. The average consumption of meat per capita a day was increased from 69.0 g in 1998 to 91.7 g in 2001. Amongst them, the consumption of pork (34.2 g) was the highest followed by that of beef (23.8 g) (18).

As the slaughtering and processing of pork meat are conducted in an exposed environment, where there is no critical step to completely remove microbial contamination, the chances of microbial contamination are very high (19, 20). In Korea, *S. aureus*, *L. monocytogenes*, and *Y. enterocolitica* have been isolated from pork slaughter and processing plants (21). Among them, *L. monocytogenes* and *Y. enterocolitica* are of major concern, due to their ability to grow at low temperatures.

Gill *et al.* (22) and Greer *et al.* (23) have previously reported that *L. monocytogenes* can grow only at 15.4°C or above on pork muscle tissue and *Y. enterocolitica* can grow above 6.4°C. Korean HACCP regulation on meat products necessitate storage of raw materials below 5°C, but, according to Rho *et al.* (21), the cold chain system in Korea was not in working condition and that was the cause for constant increase in the microbial contamination during meat distribution. Therefore, it is necessary to investigate

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the growth of psychrotrophic pathogens during pork distribution and the possibilities of microbial growth below 5°C.

Therefore, this study was intended to examine and predict the growth of *L. monocytogenes* and *Y. enterocolitica* in pork during storage conditions, and the results were compared with the results of previous reports using liquid model system for their validation in a real food system.

Materials and Methods

Microorganisms *L. monocytogenes* (ATCC 19111) and *Y. enterocolitica* (ATCC 27729) were purchased from Korea Center for Disease Control and Prevention. The microorganisms were transferred to 5 mL tryptic soy broth (TSB; Difco Laboratories, Detroit, USA) and incubated for 20 hr at 25°C. A 1 mL aliquot of both the microbial stationary phase cultures was inoculated into 100 mL TSB [0.6% (w/v) yeast extract (Difco Laboratories, Detroit, USA) was added for *L. monocytogenes*] and then incubated for 7 hr at 25°C to obtain mid-log phase cultures.

Bacterial growth on pork Pork loin was removed from the pig carcasses after 24 hr post-slaughter at a slaughterhouse located in Kyunggi-Do, Korea. The outer surface of loin was sterilized by immersion in 99% (v/v) ethanol and then the residual ethanol present on the surface was burnt off (24). After the meat surface was removed under sterile conditions, the meat was cut into pieces of 8.0 ± 0.5 g in weight and 5 mm in thickness. To confirm the sterility of the meat samples, three non-inoculated meat pieces were analyzed for the aerobic plate counts as in accordance with the Bacteriological Analytical Manual (25).

Inoculates were prepared by the dilution of 0.1 mL of a log phase culture of *L. monocytogenes* or *Y. enterocolitica* in 1 L of 0.1% peptone water. Meat pieces were immersed in the inoculate for 1 min and dried for 15 min with a sterile cellulose cloth. After inoculation, meat pieces were placed in petri plates (three pieces per plate). The plates were then wrapped with PE film with an oxygen transmission rate of about 35,000 cc/m^2/24 hr/atm and stored at 5, 10, and 15°C in an incubator (Sanyo Electric Co., Ltd., Osaka, Japan). At regular intervals of time appropriate for the incubation temperature, three pieces were removed for bacterial enumeration.

Each meat piece was 10-fold diluted in 0.1% peptone water with automatic dilutor (IUL Inc., Barcelona, Spain) and homogenized using a Stomacher (IUL Inc.). After a serial, 10-fold dilution of the homogenate in 0.1% peptone water, 100 μL aliquots of appropriate dilutions were surface plated on oxford agar (Difco Laboratories, Detroit, USA) for *L. monocytogenes* or *Yersinia* selective agar (Merck KGaA, Darmstadt, Germany) for *Y. enterocolitica*, and incubated at 30°C. The colonies were enumerated after 48 hr in the case of *L. monocytogenes* or 24 hr for *Y. enterocolitica*. The whole experiments were repeated twice.

Plate counts were transformed to $\log_{10}$ values and growth curves were generated by fitting the Gompertz function (26) to the plate count data using Origin v.6.0 (Microcal Software, Inc., Northampton, USA). Lag phase duration (LPD), exponential growth rate (EGR), generation time (GT), and maximum population density (MPD) were calculated from the Gompertz parameter A, C, B, and M values (Table 1).

Table 1. Equations for Gompertz function and derived growth kinetics values

Gompertz Function:

$L(t) = A + C \exp[-\exp\{-B(t - M)\}]$

where:

L(t) = Log count of bacteria at time t (logCFU/g)
A = Asymptotic log count of bacteria as time decreases indefinitely (i.e., initial level of bacteria) (logCFU/g)
C = Asymptotic amount of growth that occurs as t increases indefinitely (i.e., number of log cycles of growth) (logCFU/g)
M = Time at which the absolute growth rate is maximal (hr)
B = Relative growth rate at M ((logCFU/g/hr)

Derived Growth Kinetics Equations:

Exponential growth rate (EGR) = BC/e (logCFU/g/hr)
Generation time (GT) = (log2)e/BC (hr)
Lag phase duration (LPD) = M - (1/B) (hr)
Maximum population density (MPD) = A + C (logCFU/g)

Surface pH and salt (NaCl) concentration of pork The surface pH and salt (NaCl) concentration of the pork samples were determined before inoculation. Surface pH was measured using a portable pH meter (Hanna Instruments, Inc., Vila do Conde, Portugal) equipped with a flat surface electrode. The NaCl concentration was determined according to the modified method of the Korean food code (27). A 20 g piece of pork meat was weighed and placed in a furnace at 550-600°C for 7 hr until white gray ash was obtained. The ash was dissolved in 100 mL H_2O and was filtered with the help of a filter paper. After adding 2-3 drops of K_2CrO_4 (Junsei Chemical Co., Ltd., Tokyo, Japan) to the filtrate, it was titrated against 0.02 N $AgNO_3$ (Yakuri Pure Chemicals Co., Ltd., Kyoto, Japan). The percentage of NaCl was calculated by using the following equation:

$$\text{NaCl concentration (\%)} = AgNO_3\ \text{mL} \times F \times 1.17 / \text{g sample}$$

where, F = Factor for $AgNO_3$ solution.

Results and Discussion

The fitted growth curves of *L. monocytogenes* (ATCC 19111) and *Y. enterocolitica* (ATCC 27729) are shown in Fig. 1, and the values of Gompertz parameters and growth kinetics are shown in Table 2. As the inoculum's level has little influence on the growth kinetics (3), it was excluded as a study parameter for this modeling study. In addition, no growth was observed in aerobic plate counts for the initial meat samples, which confirmed the sterility of the meat samples. The growth rate of *L. monocytogenes* and *Y. enterocolitica* increased as the incubation temperature increased, as expected. However, the fact that both the pathogens did grow at 5°C on pork meat was quite different from the reports of Gill *et al.* (22) and Greer *et al.* (23), where they reported that *L. monocytogenes* and *Y.*

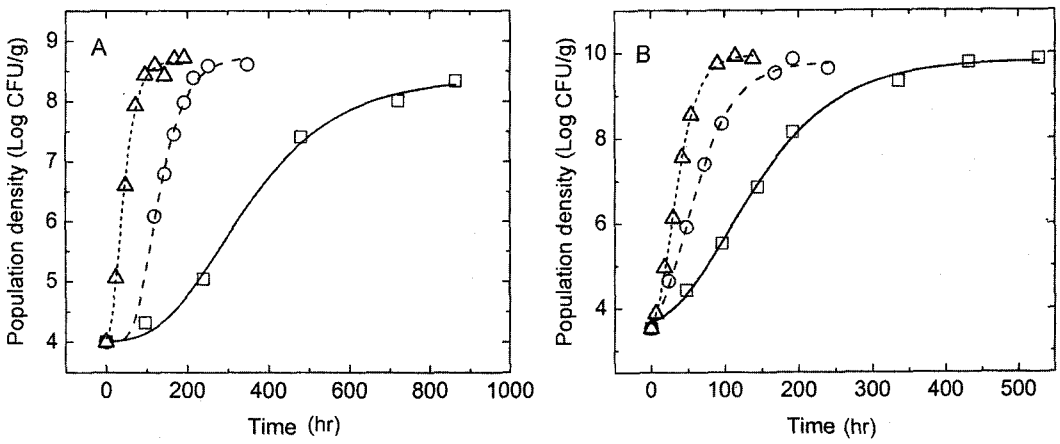

Fig. 1. Effect of storage temperature on the growth kinetics of *L. monocytogenes* ATCC 19111 (A) and *Y. enterocolitica* ATCC 27729 (B) on pork at 15°C (△), 10°C (○) and 5°C (□). Growth curves were generated using "best fit" Gompertz curve.

Table 2. Effect of storage temperature on the values of the Gompertz parameters A, C, B and M, growth kinetics values, exponential growth rate (EGR), generation time (GT), lag phase duration (LPD) and maximum population density (MPD) for aerobic cultures of *L. monocytogenes* (ATCC 19111) and *Y. enterocolitica* (ATCC 27729) on pork meat

Micro-organism	Temp. (°C)	A (logCFU/g)	C (logCFU/g)	B (logCFU/g/hr)	M (hr)	EGR (logCFU/g/hr)	GT (hr)	LPD (hr)	MPD (logCFU/g)
L. monocytogenes ATCC 19111	5	4.00	4.38	0.0065	285.3	0.010	28.74	131.45	8.38
	10	4.00	4.74	0.0220	112.8	0.038	7.85	67.35	8.74
	15	4.00	4.67	0.0436	34.1	0.075	4.02	11.16	8.67
Y. enterocolitica ATCC 27729	5	3.53	6.31	0.0126	105.7	0.029	10.29	26.33	9.84
	10	3.53	6.26	0.0276	46.0	0.064	4.74	9.77	9.79
	15	3.53	6.43	0.0510	27.1	0.121	2.50	7.49	9.96

enterocolitica can grow only at 15.4°C or above and above 6.4°C on pork muscle tissue, respectively.

As the storage temperature increased, the exponential growth rates (EGRs) increased, but the generation times (GTs) and lag phase durations (LPDs) decreased. The maximum population density (MPD) was not influenced by the storage temperature. These results were similar to the reports of Gibson *et al.* (7) and Buchanan *et al.* (12). At the same temperature, *Y. enterocolitica* (ATCC 27729) grew faster than *L. monocytogenes* (ATCC 19111) on pork.

Hence, it is evident that *L. monocytogenes* or *Y. enterocolitica* can grow on pork at low temperature during processing and distribution. Therefore, complete preventive measures are required to reduce the contamination of pork by these pathogens from the initial stage of processing.

As the intrinsic parameters of food are important for the comparison of the predictive results of this study with previous data on the same pathogens, the surface pH and the concentration of NaCl of the pork samples were measured, and they were found to be 5.80 ± 0.0637 and 0.026 ± 0.0048%, respectively. There were no wide variations in the data when compared to those of previous predictive models; however, GT values of the present study (using real pork) appeared to be greater than the previous ones. The reason for this could be explained on the basis that, the previous predictive studies have used liquid culture models, which have higher water activities than real food surface (Table 3).

In the case of LPD, there were wide variations among the models. Especially, the LPD of *L. monocytogenes* appeared to be greater and that of *Y. enterocolitica* appeared to be lower than the previous models (Table 3). This finding suggests that in addition to the issue of using real pork or artificial liquid cultures, there were other factors which affect each other and consequently influence the LPD values. McDonald *et al.* (16) have also mentioned the difficulty of measuring lag phase duration time when compared with the GT, due to the influence of environmental factors.

To summarize, the effect of temperature on the growth of *L. monocytogenes* and *Y. enterocolitica* (the frequent isolates of pork), was measured on real pork to predict the growth of both the pathogens during processing and distribution. The generation time (GT) of *L. monocytogenes* at 5, 10, and 15°C was 28.74, 7.85, and 4.02 hr, respectively, and for that of *Y. enterocolitica,* was 10.29, 4.74, and 2.50 hr, respectively. These values were greater than the GT values from previous predictive data, where liquid culture was employed instead of real pork, thus suggesting that the growth in a real food system is slower than an artificial liquid system. Hence, if the predictive models developed with liquid system are used for risk analysis for real foods, the results may deviate slightly from the real contamination level; however, there would be no problem to use the models partly as more tight levels are needed for distribution.

As both *L. monocytogenes* and *Y. enterocolitica,* appear to grow on pork at 5°C and above, it is necessary to set up preventative measures for the reduction of both the pathogens from the initial stages of pork processing. In the

Table 3. Comparison of generation time and lag phase duration of various models for aerobic cultures of *L. monocytogenes* and *Y. enterocolitica*

Micro-organism	Temp. (°C)	Generation time (hr)					Lag phase duration (hr)				
		Model 1[1)]	Model 2[2)]	Model 3[3)]	Model 4[4)]	Model 5[5)]	Model 1	Model 2	Model 3	Model 4	Model 5
L. monocytogenes	5	28.74	18.38	14.0	12.47	NM[6)]	131.45	74.6	78.4	68.44	NM
	10	7.85	6.46	5.6	4.72	NM	67.35	29.0	34.5	29.22	NM
	15	4.02	2.76	2.5	1.99	NM	11.16	13.8	16.7	14.05	NM
Y. enterocolitica	5	10.29	8.62	8.5	NM	6.9	26.33	25.9	56.0	NM	51.3
	10	4.74	3.70	3.7	NM	3.4	9.77	12.5	22.8	NM	19.0
	15	2.50	1.90	1.9	NM	1.7	7.49	7.0	11.0	NM	8.5

[1)]Present study (*Yersinia enterocolitica* (ATCC 27729) and *Listeria monocytogenes* (ATCC 19111) grown on pork meat (pH 5.8, NaCl 0.026%)).
[2)]Food MicroModel (pH 5.8, NaCl 0.0%).
[3)]USDA Pathogen Modeling Program (pH 5.8, NaCl 0.5%).
[4)]Buchanan, *et al.* (12) (pH 5.8, NaCl 0.5%).
[5)]Bhaduri, *et al.* (8) (pH 5.8, NaCl 0.5%).
[6)]NM, not measured.

future, similar trend of studies with different strains and food items have to be carried out, and the results should be compared to verify the predictive models that will allow us to set up more practical standards on storage conditions and distribution patterns of food stuffs.

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