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Studying the Effect of Temperature on Microbial Growth Using Multiplicative Model

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ABSTRACT

The specific growth rates of *Brochothrix thermosphacta, Listeria monocytogenes, Yersinia enterocolitica, Bacillus cereus, Escherichia coli* O157:H7, *Salmonella spp., Staphylococcus aureus* and *Clostridium perfringens* at various temperatures were taken from the Food MicroModel™ database, and the data sets of specific growth rate versus temperature were fitted using the multiplicative model ($r = a\,T^d$, r = specific growth rate; T = temperature; a, d = regression parameters). The exponential d-value derived from microbial growth at suboptimum temperatures reflected the effectiveness of temperature in enhancing growth. A microorganism with a large d-value exhibited a large increment of growth rate as temperature increased. The d-value of a microorganism was related to the temperature range for growth. The temperature range for the growth of psychrotrophs was usually narrow for *B. thermosphacta* and *Y. enterocolitica*; hence the d-values of these two psychrotrophs were close to 1 whereas d-values of mesophiles, such as *B. cereus, E. coli* O157:H7, *Salmonella* spp., and *S. aureus*, were 2.31~2.90, and the d-value of *C. perfringens*, a thermophile, was 3.29. The values of parameter a of the model were affected by extra salt added into cultures. For all the strains mentioned above, the *a*-values decreased when the cultures contained higher levels of salt. The lowering of the a-value implied that the influence of temperature on the growth rate in the model was reduced. The change of the d-value was dependent on the capability of the microorganism to overcome the obstacle to growth and was affected by the composition of the nutrients and by inhibitory factors in the culture. The influence of environmental factors on the d-value was also found in Chinese sausages. The d-value of a dominant spoilage strain of *Enterococcus* sp. derived from sausages was 0.833 whereas the d-value derived from MRS cultures was 2.36. In refrigerated foods which usually contained some preservatives and were stored at low temperature, the d-value of psychrotrophic spoilage bacteria was around 1. In this case, the linear model could be a reasonable choice for predicting the proliferation of spoilage bacteria.

Key Words: specific growth rate; model; predict.

I. Introduction

The Arrhenius equation has long been used to describe the relationship between microbial growth and environmental temperature. However, this equation does not adequately describe the effect of temperature on bacterial growth since the equation was originally proposed to describe the temperature dependence of the specific reaction rate constant in a chemical reaction. Ratkowsky *et al.* (1982) proposed another model which related the square root of the bacterial growth rate to temperature. This model has been proved to be preferable to the Arrhenius equation in predicting the microbial growth rate at various temperatures (Ratkowsky *et al.*, 1991; Philips and Griffith, 1987). Besides the square root model , a linear equation suggested early by Spencer and Baines (1964), has been shown to adequately predict the spoilage of chilled cod. McMeekin *et al.* (1987) noted that the square root and linear equation are special cases of the Belehradek temperature function. The Belehradek function is:

$$r = a\,(T - T_0)^d,$$

r = growth rate,

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T = temperature,

a, T_0, d = constants.

This equation is a solution of the differential equation:

$d\,r/\,d\,T = r\,d\,/\,(T - T_0)$.

This equation states that the rate at which the growth rate changes with temperature is proportional to the growth rate and inversely proportional to the difference between T and T_0. This equation has been used to describe many different biological processes (McMeekin, *et al.*, 1987). The Belehradek function leads to the square root equation when d = 2 and to the linear equation when d = 1, and the exponential d-value does not have to be an integer. Actually, Li and Torres (1993) showed that the best fit d-values for the growth of *Brochothrix thermosphacta* in media adjusted with NaCl to a_w values of 0.968, 0.950, and 0.944 were 2, 1.4 and 1, respectively. This implies that the d-value may be an index which reflects the extent of microorganism response to temperature variation.

In order to study the temperature effect on microbial growth, the Belehradek function was simplified to the multiplicative model, $r = a\,T^d$, since the constant T_0 was not changeable with temperature variation (Ratkowsky *et al.*, 1983). The multiplicative model was used to analyze the correlation of the exponential d-value with the effect of temperature on microbial growth. Eight strains of foodborne pathogens or spoilage bacteria were employed as the research subjects. The growth rate of these bacteria was acquired using the Food MicroModel™ database (McClure *et al.*, 1994). In addition, two spoilage bacteria isolated from Chinese sausage were also studied, and the growth rate was derived from growth curves measured in our laboratory.

II. Materials and Methods

From the Food MicroModel™ database, specific growth rates of *Brochothrix thermosphacta, Listeria monocytogenes, Yersinia enterocolitica, Bacillus cereus, Escherichia coli* O157:H7, *Salmonella spp., Staphylococcus aureus*, and *Clostridium perfringens* at various temperatures were acquired. The identities of these bacteria are shown in Table 1.

A strain of *Leuconostoc mesenteroides* (Hwang, 1997) and a strain of *Enterococcus* sp. (Hung, 1995) were isolated from Chinese sausage in our laboratory as experimental subjects. To measure the growth curve of *L. mesenteroides*, 250 ml flasks containing 100 ml TSB (tryptic soy broth (DIFCO Co.)) were inoculated with seed culture which had been grown in the same media for 18 hours at 25°C. To measure the growth of *Enterococcus* sp., MRS broth (Lactobacilli MRS broth (DIFCO Co.))was used. The initial OD_{600nm} reading of the cultures was adjusted to around 0.01~0.02. The inoculated flasks were incubated at various temperatures on an orbital shaker (120 rpm).

Table 1. Characteristics of Bacterial Strains.

Species	Gram's staub	Shape	Spore	Optimal growth temperature (°C)	Maximum NaCl(%)
Brochothrix thermosphacta	G +	rod	non	20~25	8
Listeria monocytogenes	G +	rod	non	20~25	11.5
Yersinia enterocolitica	G –	rod	non	3~15	8
Bacillus cereus	G +	rod	spore	28~35	6.5
Escherichia coli O157:H7	G –	rod	non	30~37	6.5
Salmonella	G –	rod	non	30~37	4.5
Staphylococcus aureus	G +	coccus	non	35~37	13.5
Clostridium perfringens	G +	rod	spore	43~45	5

(Food MicroModel™,1996)

Growth was measured by monitoring cell turbidity (OD_{600nm}) using a Spectronic 20 (MILTON ROY, USA), and triplicate data were obtained. Growth curves of *Enterococcus* sp. at various temperatures in sausage were determined using the standard plate count method. The specific growth rate was calculated from the slope of the line when the organisms grew exponentially.

III. Results and Discussion

1. The Correlation of the Exponential D-value with the Temperature Effect on Microbial Growth

The specific growth rates of eight bacterial strains at various temperatures were taken from the Food MicroModel™ database. The data sets of specific growth rate versus temperature were fitted to the multiplicative model (Microsoft Excel for Windows 95) and ploted in Figs. 1 and 2. The specific growth rate increased as the incubation temperature increased. The increment of specific growth rate correlated with the d-value. A strain with a greater d-value had a larger increment of specific growth rate as the temperature increased. The average exponential d-value of the psychrotrophs was less than 2. Actually, the d-values of *B. thermosphacta* and *Y. enterocolitica* were close to 1, and their regression lines were linear. The d-values of mesophiles were in the range of 2.32-2.90 (Fig. 2), and that of the only thermophile, *C. perfringens*, was larger than 3. The d-values and the increment of specific growth rate followed the order of psychrotroph < mesophile < thermophile (Fig. 1). This order implies that the effect of temperature on the growth of the psychrotrophs was less than that on the growth of the mesophiles and thermophiles.

We thought that the broadness of the temperature range of the growth data might affect the exponential d-values. Actually, the d-values were affected by the suboptimum temperature of the bacteria. The range of the suboptimum temperature of *B. thermosphacta* and Y. *enterocolitica* was smaller than that of the other bacteria as shown in Table 1.

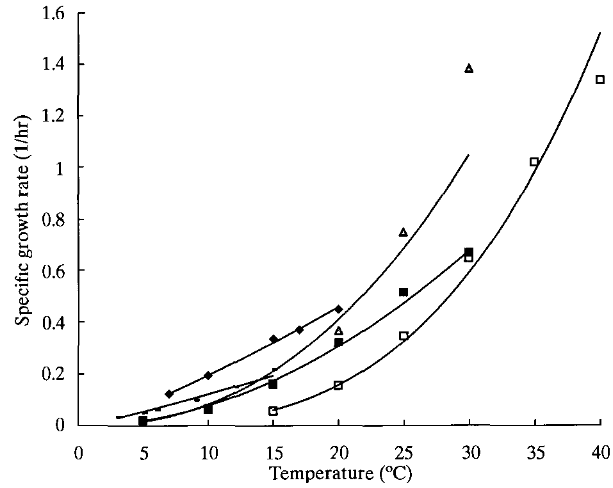

Fig. 1. Multiplicative plots of specific growth rate vs temperature for *Brochothrix thermosphacta, Listeria monocytogenes, Yersinia enterocolitica, Bacillus cereus and Clostridium perfringens.*
◆ *Brochothrix thermosphacta* ($r = 0.0107T^{1.25}$; $R^2 = 0.997$)
■ *Listeria monocytogenes* ($r = 0.0008T^{1.96}$; $R^2 = 0.994$)
– *Yersinia enterocolitica* ($r = 0.0088T^{1.14}$; $R^2 = 0.978$)
Δ *Bacillus cereus* ($r = 0.0004T^{2.32}$; $R^2 = 0.972$)
□ *Clostridium perfringens* ($r = 8E\text{-}6T^{3.29}$; $R^2 = 0.995$)

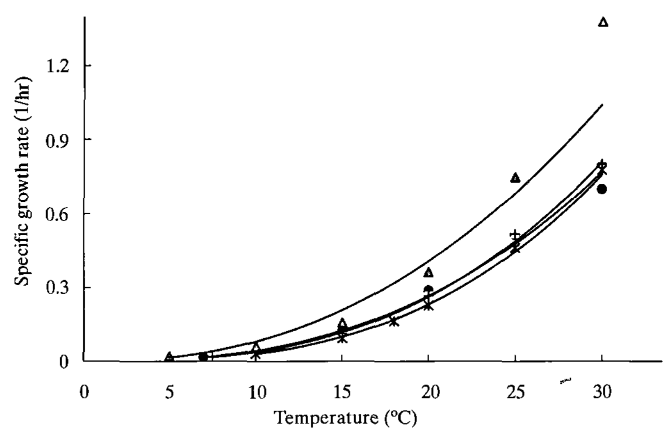

Fig. 2. Multiplicative plots of specific growth rate vs temperature for mesophiles.
Δ *Bacillus cereus* ($r = 0.0004T^{2.32}$; $R^2 =0.972$)
✶ *Escherichia coli* O157:H7 ($r = 4E\text{-}5T^{2.90}$; $R^2 = 0.998$)
• *Salmonella* spp.($r = 0.0001T^{2.60}$; $R^2 = 0.997$)
+ *Staphylococcus aureus* ($r = 7E\text{-}5T^{2.78}$; $R^2 = 0.998$)

2. The Influence of Extra NaCl in Media on the Multiplicative Model

According to Table 2, the variation of the regression coefficients (a-values) of the model showed a tendency of the coefficients to become smaller as the concentration of NaCl in the media increased. The tendency of the a-values to become smaller meant that the temperature effect of boosting the growth of microorganisms in extra NaCl became weaker. However, the variation of the exponential d-value of the model did not show the same tendency. Actually, the d-values of *B. thermosphacta*, *L. monocytogenes*, *E. coli* O157:H7, and *B. cereus* became smaller as the concentration of NaCl in the media increased whereas the d-values of *S. aureus* and *Y. enterocolitica* became larger as the concentration of NaCl in the media increased. The variation of the d-values of

Table 2. Multiplicative Models Derived from the Cultures Containing Various Amounts of Salt

Species		NaCl concentration (%)				
Brochothrix thermosphacta		0	2.0	4.0	6.0	8.0
	Equation	$r = 0.0107T^{1.25}$ $R^2 = 0.997$	$r = 0.0101T^{1.19}$ $R^2 = 0.996$	$r = 0.0092T^{1.12}$ $R^2 = 0.995$	$r = 0.008T^{1.06}$ $R^2 = 0.993$	$r = 0.0066T^{1.00}$ $R^2 = 0.990$
Listeria monocytogenes		0	3.0	6.0	9.0	11.5
	Equation	$r = 0.0008T^{1.96}$ $R^2 = 0.994$	$r = 0.0009T^{1.90}$ $R^2 = 0.994$	$r = 0.0007T^{1.83}$ $R^2 = 0.995$	$r = 0.0004T^{1.77}$ $R^2 = 0.994$	$r = 0.0002T^{1.67}$ $R^2 = 0.994$
Yersinia enterocolitica		0	2.0	4.0	6.0	8.0
	Equation	$r = 0.0088T^{1.13}$ $R^2 = 0.977$	$r = 0.0061T^{1.16}$ $R^2 = 0.997$	$r = 0.0031T^{1.17}$ $R^2 = 0.971$	$r = 0.0009T^{1.25}$ $R^2 = 0.985$	$r = 0.0003T^{1.14}$ $R^2 = 0.824$
Bacillus cereus		0	1.5	3.0	4.5	6.5
	Equation	$r = 0.0004T^{2.31}$ $R^2 = 0.972$	$r = 0.0004T^{2.23}$ $R^2 = 0.975$	$r = 0.0004T^{2.11}$ $R^2 = 0.973$	$r = 0.0003T^{2.01}$ $R^2 = 0.975$	$r = 1E-4T^{2.21}$ $R^2 = 0.996$
Escherichia coli O157:H7		0	1.5	3.0	4.5	6.5
	Equation	$r = 4E-5T^{2.90}$ $R^2 = 0.998$	$r = 4E-5T^{2.89}$ $R^2 = 0.998$	$r = 3E-5T^{2.89}$ $R^2 = 0.998$	$r = 2E-5T^{2.87}$ $R^2 = 0.998$	$r = 8E-6T^{2.86}$ $R^2 = 0.998$
Salmonella spp.		0	1.0	2.0	3.0	4.5
	Equation	$r = 0.0001T^{2.60}$ $R^2 = 0.997$	$r = 0.0001T^{2.62}$ $R^2 = 0.997$	$r = 1E-4T^{2.63}$ $R^2 = 0.997$	$r = 7E-5T^{2.67}$ $R^2 = 0.993$	$r = 5E-5T^{2.66}$ $R^2 = 0.997$
Staphylococcus aureus		0	3.0	6.0	9.0	13.5
	Equation	$r = 7E-5T^{2.76}$ $R^2 = 0.998$	$r = 4E-5T^{2.87}$ $R^2 = 0.997$	$r = 2E-5T^{2.98}$ $R^2 = 0.997$	$r = 8E-6T^{3.21}$ $R^2 = 0.999$	$r = 2E-6T^{3.55}$ $R^2 = 0.999$
Clostridium perfringens		0	2.0	3.0	4.0	5.0
	Equation	$r = 8E-6T^{3.29}$ $R^2 = 0.998$	$r = 8E-6T^{3.30}$ $R^2 = 0.995$	$r = 7E-6T^{3.30}$ $R^2 = 0.995$	$r = 6E-6T^{3.30}$ $R^2 = 0.995$	$r = 5E-6T^{3.29}$ $R^2 = 0.995$

Salmonella spp. and *Clostridium perfringens* was ambiguous. The results indicated that the d-values might have been related to the adaptability of the microorganisms to extra NaCl added in media.

3. The Variation of the Exponential D-value of the Multiplicative Model Under Different Conditions

A strain of *Leuconostoc mesenteroides*, which was isolated from commercial Chinese sausage was used to study the variation of the d-values derived from different cultures (Fig. 3). Under growth in TSB broth, the d-value of *L. mesenteroides* was 1.73, and the d-value derived from the MRS culture was 1.82. The d-value derived from the MRS culture was higher than that derived from the TSB culture. The reason for this might have been that the nutrients of the MRS broth were richer than that of TSB.

It was observed (Fig. 3) that the d-value derived from the MRS culture contained additives; potassium sorbate (0.2%), sodium nitrite (0.012%), and

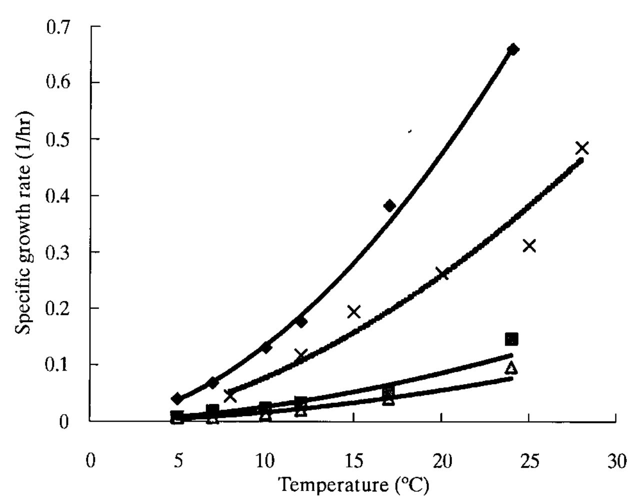

Fig. 3. Multiplicative plots of specific growth rate vs temperature for *Leuconostoc mesenteroides* derived from different media.
◆ MRS ($r = 0.002\ T^{1.82}$; $R^2 = 0.997$)
+ TSB ($r = 0.0014\ T^{1.73}$; $R^2 = 0.945$)
■ MRS + additives ($r = 0.0005\ T^{1.79}$; $R^2 = 0.958$)
▲ MRS + additives + 1% agar ($r = 0.0003\ T^{1.76}$; $R^2 = 0.955$)

sodium chloride (2%) decreased to 1.79. The lower d-value meant that the additives affected the growth of the bacteria (Jay, 1996) and reduced the temperature effect on overcoming the growth obstacles caused by additives. After 1% agar was added to the modified MRS medium to obtained a semi-solid condition which would restrain the diffusion of nutrients and harmful metabolites, it was found that the growth of bacteria was suppressed, and that the d-value derived from this semi-solid culture was reduced to 1.76. This result might explain the observation that the d-value of *Enterococcus* sp. in sausage was reduced (Fig. 4) since the diffusion of nutrients and toxic metabolites was restrained in the semi-solid agar. It became clear (Fig. 3) that the nutrient content of the media was an important factor in determining d-values, and that the growth hurdles created by physicochemical factors reduced the d-values.

4. The Correlation of Exponential D-values to the Appropriateness of Belehradek Models

A strain of *Enterococcus* sp.was isolated as a dominant spoilage microorganism in home-made Chinese sausage (Hung, 1995). The specific growth rates of the bacterium at 8, 10, 15, 20, and 25°C in sausage were measured . It was found that the best growth-temperature relationship for this strain in sausage followed the linear model, r = 0.321 + 0.0253T (R^2 = 0.84). Using the same method, the best growth-temperature relationship for this strain in MRS broth was determined as $r^{1/2} = -0.0757 + 0.0358\,T$ ($R^2 = 0.99$). The data was also fitted using the multiplicative model, and the exponential d-values derived from sausage and MRS broth were o.833 and 2.36, respectively (Fig. 4). It is interesting to note that for bacteria grown in MRS, the rich nutrient media, the growth-temperature relationship followed the square root model, and the exponential value of the multiplicative model was around 2. When bacteria grew in sausage , a poorer media compared with MRS, the growth-temperature relationship followed the linear model, and the d-value of the multiplicative model approached 1.

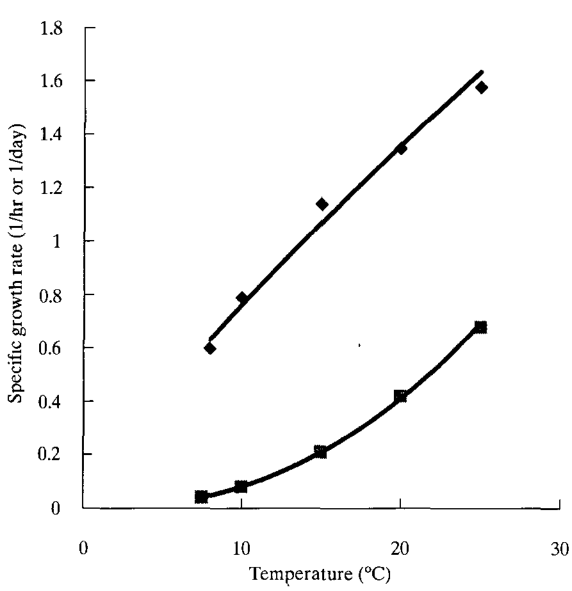

Fig. 4. Multiplicative plots of specific growth rate vs temperature for *Enterococcus* sp. derived from MRS cultures and sausages.
◆ for sausages ($r = 0.1121\ T^{0.833}$, $R^2 = 0.9848$)
■ for MRS cultures ($r = 0.0003\ T^{2.36}$, $R^2 = 0.9998$)

IV. Conclusion

The expontential d-value of the multiplicative model might be an intrinsic parameter indicating the response of a microorganism to changing temperature. The d-values in this study revealed that the temperature effect enable a microorganism to overcome growth obstacles . The d-values derived in the suboptimum temperature range might be useful as a reference for the classification of psychrotrophs, non-psychrotroph mesophiles, and thermophiles. For certain psychrotrophs, such as *B. thermosphacta* and *Enterococcus* sp., the d-values approached 1 in restrained cultures due by physicochemical factors. Spoilage bacteria in refrigerated meat products, such as sausages which usually contain some preservatives, could not grow in a refrigerator. In these products, the d-values of the dominant spoilage psychrotrophs , *B. thermosphacta* and *Enterococcus* sp., approached 1. Therefore, the linear model was appropriate to describe the growth-temperature relationship. It is concluded that to predict the growth of spoilage psychrotrophs in chilled meat products or refrigerated foods, the linear model is a reasonable choice.

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Nomenclature

r	specific growth rate, $hour^{-1}$
T	temperature, °C
T_0	a hypothetical temperature as 'biological zero', °C
a, d	fitted parameters of the multiplicative model, dimensionless
a_w	water activity, dimensionless

References

Food MicroModel Ltd. (1996) Technical data sheet, In: *FoodMicroModel User Mannual* (version 2), pp.25-81. Surry,UK.

Hung, M.J. (1995) Studies on the application of predictive microbiology to monitor the qualitative variation of refrigerated foods in distribution chain. Master thesis. Graduate Institute of Food Science. Tung Hai University, Taichung, Taiwan, R.O.C.

Hwang, C.C. (1997) Studying the effect of temperature on microbial growth in different media by multiplicative model. Master thesis. Graduate Institute of Food Science. Tung Hai University, Taichung, Taiwan, R.O.C.

Jay, J.M. (1996) Food preservation with chemicals. In : *Modern Food Microbiology*. pp.273-303. New York, NY.

Li, K.-Y. and Torres, T.A. (1993) Microbial growth estimation in liquid media explosed to temperature. *Journal of Food Science*, **58:**265-275.

McClure, P.J., Blackburn, C. deW., Cole, M.B., Curtis, P.S., Jone, J.E. and Roberts, T.A. (1994) Modelling the growth, survival and death of microorganism in foods: the UK Food MicroModel approach. *International Journal of food microbiology*, **23:**265-275.

McMeekin, T.A.,Chandaler, R.E. and Ratkowsky, D.A. (1987) Model for combined effect of temperature and salt concentration/ water activity on the growth rate of *Staphylococcus xylosus*. *Journal of Applied Bacteriology*, **62:**543-550.

Phillips, J.D. and Griffiths, M.W. (1987) The relationship between temperature and growth of bacteria in dairy products. *Food Microbiology*, **4:**173-185.

Ratkowsky, D.A., Olley, J., McMeekin, T.A. and Ball, A. (1982) Relationship between temperature and growth rate of bacterial cultures. *Journal of Bacteriology*, **149:**1-5.

Ratkowsky, D.A., Lowry, R.K., McMeekin, T.A., Stokes, A.N. and Chandaler, R.E. (1983) Model for bacterial culture growth rate throgh out the entire bikinetic temperature range. *Journal of Bacteriology*, **154:**1222-1226.

Ratkowsky, D.A., Ross, T., McMeekin, T.A. and Olley, J. (1991) Comparison of Arrhenius-type and Belehradek-type models for prediction of bacterial growth in foods. *Journal of Applied Bacteriology*, **71:**452-456.

Spencer, R. and Baines, C.R. (1964) The effect of temperature on the spoilage of wet white fish. *Food Tecnology*, **18:**769-773.

以乘冪模式探討溫度對微生物生長的影響

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摘　要

本研究從Food MicroModel™ Database中擷取*Brochothrix thermosphacta*、*Listeria monocytogenes*、*Yersinia enterocolitica*、*Bacillus cereus*、*Escherichia coli* O157:H7、*Salmonella spp.*、*Staphylococcus aureus*及*Clostridium perfringens*八株菌不同溫度下之比生長速率，將溫度與其相對的比生長速率契合入乘冪模式（$r = aT^d$；r = 比生長速率；T = 溫度；a,d = 迴歸參數），並以此模式探討溫度對微生物生長速率的影響。模式中指數d-值在次最適(suboptimum)生長溫度範圍內可以反映溫度對生長速率變化的影響力，d-值愈大溫度上升時生長速率增加愈多。由數據顯示d-值與微生物生長溫度範圍有關。生長溫度範圍狹窄者，d-值小，如耐冷菌*B. thermosphacta*與*Y. enterocolitica*，其d-值均趨近1。中溫菌*B. cereus*，*S. aureus*，*Salmonella* sp.，*E. coli* O157:H7，d-值在2.31~2.90間。高溫菌*C. perfringens* d-值是3.29。乘冪模式中的係數*a* 值會因限制因子的存在而減小，表示在不良環境下溫度對菌體生長的影響力變弱。在不良環境下不同菌株的d-值，有的會增加，有的會減少。因此d-值與菌株本身克服生長障礙的能力，以及培養基的營養組成份和限制因子有關。環境影響d-值的現象亦可在中式香腸中觀察到。*Enterococcus* sp.生長在冷藏香腸的d-值是0.833，而在MRS的d-值是2.36。冷藏食品通常含有多種添加物，因此於其中的耐冷腐敗菌，d-值會接近1，所以預測在此環境下耐冷腐敗菌的增殖，線型模式是合理的選擇。