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Comparison of Arrhenius-type and Bělehrádek-type models for prediction of bacterial growth in foods

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D.A. RATKOWSKY, T. ROSS, T.A. MCMEEKIN AND J. OLLEY. 1991. The development of Arrhenius-type ('Schoolfield') and Bělehrádek-type (square root) models that describe microbial growth rates is briefly described. Both types of model have been advocated for use in predictive microbiology. On the basis of published data sets for the growth of bacteria, the consequences of mathematical transformation of data and the use of invalid stochastic assumptions upon model predictions are demonstrated. Mean square error is shown to be an inappropriate criterion by which to compare the performance of predictive models. The data show that bacterial growth responses such as generation time and lag time become more variable as their mean magnitude increases. The practical consequences of such variability for predictive microbiology are discussed.

INTRODUCTION

Predictive microbiology is the term used to describe the mathematical modelling of the effects of temperature, water activity, pH and other environmental influences on the rate of microbial growth or the time to reach specified numbers of micro-organisms or their metabolites. The concept has been reviewed by a number of authors (Farber 1986; Baird-Parker & Kilsby 1987; Gould 1989; Roberts 1990). The potential benefits of predictive food microbiology depend entirely on the selection of appropriate models to describe the effects of environmental factors on microbial growth rate. Two types of kinetic model, *viz.* non-linear Arrhenius and Bělehrádek-type models, have gained prominence in attempts to describe the effects of temperature and other environmental factors on lag phase duration and growth rate. Arrhenius-type models are typically applied with the dependent variable expressed as ln *rate*. The Bělehrádek-type model most used in predictive microbiology, the square-root model, expresses the dependent variable as $\sqrt{rate}$. The development of these models is described in the Appendix. This paper describes the effects of the form of the model on the variance in rate and time for Arrhenius-type and square-root models.

Adair *et al.* (1989) published the first direct comparison of Arrhenius-type models, for which they expressed the dependent variable as ln *time*, and Bělehrádek-type models. Specifically, they compared a six-parameter form of equation A3 with equation A7, and a four parameter form of equation A1 (or equation A2) with equation A6. They referred to the models as 'Schoolfield' models. Their conclusions were commented on by Davey (1989), McMeekin *et al.* (1989) and Kilsby (1989). McMeekin *et al.* (1989) criticized the approach of Adair *et al.* (1989) because small data sets were used to compare models with different numbers of parameters and because the fitted models were not validated against other data sets to test their general applicability. In addition, unusual values of the thermodynamic constants of the 'Schoolfield' models were obtained for several data sets which suggested that a relationship unique to a particular data set had been obtained. On the basis of the protocol adopted by Adair *et al.* (1989) it is not surprising that the 'Schoolfield' models, which have two additional fitted parameters, appeared to outperform the square root models.

Comparison of the models by Adair *et al.* (1989) was based upon the mean squared error (MSE) between the observed generation or lag time and that predicted by the respective models. More detailed examination of the effects of mathematical transformations of the observed times and model predictions on the magnitude of MSE, and of the stochastic assumptions inherent in fitting the competing models to data, led to the hypothesis that transformation effects and inappropriate stochastic assumptions contributed to the apparent superiority of the predictions of the 'Schoolfield' models when compared with those of the square root model.

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We here present the results of our evaluation of these effects based on theoretical considerations and supported by published data sets, and we make further comparisons between the square root and Arrhenius-type models.

MATERIALS AND METHODS

To demonstrate the effect of mathematical transformation of data the simple square root model (equation A6) was expressed with time, ϕ, rather than rate as the dependent variable. Upon transformation equation A6 becomes the non-linear equation:

$$\phi = 1/k = 1/[b(T - T_{min})]^2 \qquad (1)$$

where k, T, T_{min} and b have the same meaning as in equation A6. Data can be fitted to this model by standard non-linear regression routines.

The effect on variance of transforming predicted values of the natural logarithm of *time*, or $\sqrt{rate}$ to generation or lag *time* was determined. The relationship between the variance on the *time* scale and the variance on the $\sqrt{k}$ scale, where the rate, k, is calculated as $1/\phi$, is approximately given by:

$$\begin{aligned}\mathrm{Var}(\sqrt{k}) &= \mathrm{Var}(1/\phi)^{1/2}\\ &= [d(1/\phi)^{1/2}/d\phi]^2\ \mathrm{Var}(\phi)\\ &= (1/4\phi^3)\ \mathrm{Var}(\phi)\end{aligned}$$

or

$$\mathrm{Var}(\phi) = 4\phi^3\ \mathrm{Var}(\sqrt{k}) \qquad (2)$$

A similar calculation to relate the variance on the *time* scale to the variance on the ln *time* scale as in the 'Schoolfield' model leads to

$$\begin{aligned}\mathrm{Var}(\ln\phi) &= [d(\ln\phi)/d\phi]^2\ \mathrm{Var}(\phi)\\ &= (1/\phi^2)\ \mathrm{Var}(\phi)\end{aligned}$$

Furthermore, since

$$k = 1/\phi$$

then

$$\begin{aligned}\ln k &= \ln(1/\phi)\\ &= -\ln\phi\end{aligned}$$

therefore

$$\mathrm{Var}(\ln k) = \mathrm{Var}(-\ln\phi) = (1/\phi^2)\mathrm{Var}(\phi)$$

or

$$\mathrm{Var}(\phi) = \phi^2\ \mathrm{Var}(\ln k) \quad \text{or} \quad \phi^2\ \mathrm{Var}(\ln\phi) \qquad (3)$$

Since Arrhenius-type models are usually fitted in the logarithmic form (i.e. the dependent variable is ln *rate*) the following discussion will be based on this transformation of the dependent variable but, as is apparent from equation 3, the discussion applies equally to variance in ln *time*.

Variance in the generation *time*, ln *rate* and $\sqrt{rate}$ was calculated for the replicated data set of M.G. Smith (personal communication) (CSIRO Division of Food Processing, Meat Research Laboratory, Cannon Hill, Australia) who generously provided his raw data for the growth of coliform organisms on meat at different temperatures, which is summarized in Smith (1985). Raw data for the growth response of *Listeria monocytogenes*, provided by Dr R.L. Buchanan (US Department of Agriculture, Agricultural Research Station, Philadelphia, USA) were similarly treated. These data are summarized in Buchanan & Phillips (1990). The variances derived were used to test (1) the validity of the predicted relationships (equations 2 and 3) between variances in various transformations of the data and (2) the validity of the stochastic assumptions inherent in the three forms of the predictive models (equations 1, A5 and A6).

RESULTS AND DISCUSSION

Minimization of MSE

Least squares modelling, in common with modelling by various other criteria, involves consideration of both the deterministic part of the model, that is the relationship between the response (dependent) variable and the explanatory (regressor or independent) variable, and the stochastic part of the model, that is the error assumption (Ratkowsky 1990).

Tables 1 and 2 present the data of Smith (1985) for the growth of coliform organisms in meat, together with predictions for these data for the four-parameter 'Schoolfield' model (equation A5 with the term containing the parameters G and H deleted) and both forms of the simple square root model (equations A6 and 1). The improvement in the square root model predictions at the lowest temperatures when equation 1 is used is marked. By the MSE criterion the two-parameter square root model performs about as well as the four-parameter 'Schoolfield' model.

A similar improvement in goodness of fit at the lowest temperatures is evident with other data sets. Table 3 shows the results of Adair *et al.* (1989) for growth of the Gram-negative spoilage microbiota in air-packed beef mince. Again, the improvement at the lowest temperatures is marked and in terms of the overall goodness of fit judged by the MSE criterion the two models perform equally well. However, the price paid for the improved precision at the lowest temperature is decreased precision at the highest temperatures, less consistency in the estimation of T_{min} and a non-random distribution of residuals.

Table 1 Observed and predicted generation times for growth of coliforms in meat [data of Smith (1985)]

	Generation time (h)			
		Square root model predictions		
Temperature (°C)	Observed time	Using eqn (A6)	Using eqn (1)	'Schoolfield' prediction
10	6·68	8·50	6·69	6·57
15	2·63	2·66	2·61	2·78
20	1·49	1·28	1·38	1·39
25	0·80	0·75	0·85	0·80
30	0·47	0·49	0·58	0·50
35	0·34	0·35	0·42	0·33
MSE		0·57	0·006	0·008
T_{min} (generation time)		3·65°C	1·67°C	

MSE, Mean square error.

The values of the constant T_{min}, estimated by equation A6 for the lag and generation time of coliforms in meat, differ by only 0·8°C, whereas those estimated by equation 1 differ by 3·0°C. Thus, to model observed but not necessarily 'representative' values at low temperatures equation 1 sacrifices an accurate determination of T_{min}.

The above exercise shows how the MSE values can be reduced by incorrect stochastic assumptions. In equation 1 an error term homogeneous in time is assumed, i.e. the magnitude of the error is assumed to be constant irrespective of the magnitude of the observed generation or lag time. As can be seen from Table 4, this is an invalid assumption and is contradicted by the data.

Stochastic assumptions

The stochastic assumption for fitting the square root model is quite different from that implicit in the 'Schoolfield' model. In the former case, the minimization procedure is carried out on a 'square root of rate' scale, which assumes that the residuals in the square root of rate have a constant variance. When these fitted values of $\sqrt{k}$ are transformed to predict lag or generation times, small discrepancies on the rate scale can become rather large discrepancies on the time scale; this is evident from Table 5. When the MSE criterion is applied to rates predicted by either model, there is no difference in the goodness of fit, yet there is a large difference in the MSE when these predictions are transformed to times. This further emphasizes that the differences reported by Adair *et al.* (1989) relate more to the stochastic assumption than to the model itself.

Table 4 and Fig. 1 show the variances of generation time (Var $[\phi_g]$), square root of generation rate (Var$[\sqrt{k_g}]$) and natural logarithm of generation rate (Var$[\ln k_g]$) as functions of generation time. The increase in Var$[\phi_g]$ and Var$[\ln k_g]$ with increasing generation time are striking. On the other hand, Var$[\sqrt{k_g}]$ is virtually constant, justifying the use of a stochastic term that is homogeneous in $\sqrt{k}$, the assumption made when equation A6, the simple square root model, is fitted.

Table 2 Observed and predicted lag times for growth of coliforms in meat [data of Smith (1985)]

	Lag time (h)			
		Square root model predictions		
Temperature (°C)	Observed time	Using eqn (A6)	Using eqn (1)	'Schoolfield' prediction
10	23·25	18·39	23·22	23·18
15	5·90	6·35	6·22	5·98
20	3·00	3·19	2·83	2·92
25	1·92	1·91	1·61	1·88
30	1·19	1·27	1·04	1·30
35	0·96	0·91	0·72	0·92
MSE		3·98	0·048	0·006
T_{min} (lag time)		2·86°C	4·64°C	

MSE, Mean square error.

Table 3 Observed and predicted lag times for the growth of Gram-negative spoilage biota in air-packed beef mince [data of Adair *et al.* (1989)]

	Lag time (h)			
		Square root model predictions		
Temperature (°C)	Observed time	Using eqn (A6)	Using eqn (1)	'Schoolfield' prediction
−2.0	165.91	222.58	166.45	158.35
0.0	98.38	114.84	103.12	107.08
2.0	85.37	69.89	70.10	73.93
5.0	36.19	39.53	43.93	44.33
10.0	20.83	19.80	24.20	21.50
13.0	20.74	14.33	18.15	15.04
17.0	8.62	9.96	13.06	10.12
20.0	7.37	7.87	10.53	7.88
25.0	5.54	5.62	7.69	5.54
30.0	4.25	4.20	5.87	4.11
MSE		377.91	37.09	36.52
T_{min}		−7.10°C	−9.40°C	

MSE, Mean square error.

When fitting the 'Schoolfield' models Adair *et al.* (1989) assumed that Var[ln *time*] is homogeneous, an assumption contradicted by the data of Smith (personal communication) in Fig. 1. A consequence of this incorrect assumption is that when the model is fitted to data by ordinary (i.e. unweighted) least squares, there is a tendency for the more variable points (i.e. those at lower temperatures) to be more influential in determining the least squares line than the points at higher temperatures. The result is a better fit to the points at low temperature. Schoolfield *et al.* (1981) recognized the problem caused by the non-homogeneity of Var[ln *k*] and performed least squares non-linear regression 'weighted according to the reciprocal of the rate values'.

The above explanation coupled with the fact that the 'Schoolfield' models have two more parameters than the square root models, are the major reasons why Adair *et al.* (1989) obtained better fits with the 'Schoolfield' models than with the square root models. The increase in variance in generation time and in lag time as the temperature moves further from the optimum, means that obtaining representative estimates becomes increasingly difficult as the population is placed under stressful conditions.

The increased variability of data poses a greater problem for the 'Schoolfield' model than for the square root model because Var[$\sqrt{k}$] is constant but Var[ln *k*] increases progressively with response time. The consequence of this is that estimates of parameter values can vary widely at low temperatures and long times; for example, the value for ΔH_L (enthalpy of low temperature inactivation of growth) is virtually determined by the data at the lowest temperatures.

This problem does not arise with the square root model because Var[$\sqrt{k}$] is homogeneous throughout the bio-

Table 4 Variance data for generation times of *Escherichia coli* on meat carcasses from the raw data of Smith (personal communication)

			Variance				
			Observed			Generation time predicted from	
Temperature (°C)	Mean generation time (h)	No. of replicates	Generation time	ln *rate*	$\sqrt{rate}$	eqn (2)	eqn (3)
10	5.86	7	0.85	0.027	0.0012	0.98	0.93
15	2.50	11	0.083	0.014	0.0014	0.090	0.087
20	1.49	7	0.012	0.0056	0.00096	0.013	0.013
25	0.79	9	0.0038	0.0052	0.0016	0.0032	0.0032
30	0.47	14	0.0019	0.0088	0.0046	0.0019	0.0020
35	0.34	8	0.00029	0.0025	0.0019	0.00029	0.00029
40	0.28	8	0.00026	0.0034	0.0030	0.00025	0.00025

Table 5 Mean square error (MSE) of rates (generations/h) predicted by the 'Schoolfield' and square root models for the data sets of Smith (1985) and Adair *et al.* (1989) presented in Tables 1, 2 and 3

		Predicted rate	
Data from	Observed rate	'Schoolfield' model	Square root model
Table 1	0·150	0·152	0·118
	0·380	0·360	0·376
	0·671	0·719	0·781
	1·250	1·250	1·333
	2·128	2·000	2·041
	2·941	3·030	2·857
MSE (*rate*)		0·004500	0·005788
Table 2	0·043	0·043	0·054
	0·169	0·167	0·157
	0·333	0·342	0·313
	0·521	0·532	0·524
	0·840	0·769	0·787
	1·042	1·087	1·099
MSE (*rate*)		0·001220	0·001125
Table 3	0·006	0·006	0·004
	0·010	0·009	0·009
	0·012	0·014	0·014
	0·028	0·023	0·025
	0·048	0·047	0·051
	0·048	0·066	0·070
	0·116	0·099	0·100
	0·136	0·127	0·127
	0·220	0·181	0·178
	0·235	0·243	0·238
MSE (*rate*)		0·000238	0·000261

MSE, Mean square error.

kinetic range. Predictive models should be based on extensive data sets and we suggest, from experience, that a minimum of 10–15 temperature/rate determinations at close temperature intervals are required to obtain reliable estimates of parameter values. In adopting this strategy the size of the experiment limits the number of replicates at any temperature particularly if plating methods, rather than turbidity or other indirect methods, are used to estimate growth. To obtain confidence limits for estimates of growth rate or lag phase duration, replicate determinations can be made at a few temperatures and the variance in real time calculated at any temperature by application of equations 2 or 3.

As further hurdles to bacterial growth such as reduced water activity, unfavourable pH, etc. are added, the variance increases further. This can be inferred from raw data provided by R.L. Buchanan and summarized by Buchanan & Phillips (1990) for growth of *L. monocytogenes*. Generally, when the generation time was less than 1·0 h, the absolute error was of the order of 0·1 or less, but for generation times of 5 h or more the variance may be as high as 4 h. This latter value was calculated for nine replicates at 5°C, pH 7·5, 5% NaCl and without nitrite under aerobic conditions. Under more stressful conditions produced by low pH (19°C, pH 4·5, 5% NaCl, without nitrite), 19 data points gave a mean generation time of 8·3 h with a variance of 25·66. Twelve points were clustered about the mean, while three values were clustered at 19 h and four were around 3·5 h. Seven replicates of lag phase duration at 19°C, pH 6·0, 5% NaCl and without nitrite varied from 2·92 to 16·12 h. Four of the estimates were clustered below the mean and three were clustered above the mean. These unusual patterns of distribution make estimation of representative mean responses difficult.

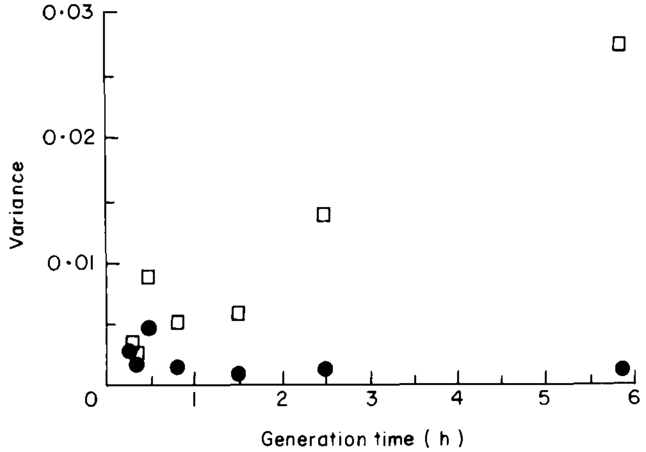

Fig. 1 Comparison of the variance in $\sqrt{(rate)}$ and the variance in ln (*rate*) for the generation time data of Smith (personal communication). Var[ln (*rate*)] = Var[ln (*time*)] see equation (3). ●, Var $\sqrt{k}$; □, Var ln (*rate*)

Under extremely stressful conditions near the limiting values for survival (low pH/high salt), Cole *et al.* (1990) have shown that the results obtained become increasingly erratic, making predictions of survival times even less reliable. The contention of Kilsby (1989), that it is most important to fit predictions to actual data obtained, can only be correct if these data very precisely approximate to the mean response. To obtain representative estimates becomes increasingly difficult at longer generation times and lag times, when the latter in particular show marked variability.

Models have been developed to describe the effect of environmental factors on the growth of spoilage and pathogenic bacteria. In both situations the lag and logarithmic phases of growth are of interest. Since spoilage results from prolific growth of organisms, the logarithmic phase dominates models for spoilage processes. Conversely, as the tolerance levels for pathogens in food are much lower, and in some cases zero, emphasis will be on lag phase modelling. In view of the increasing variability of estimates under severe growth-limiting conditions, completely accurate predictions cannot be made from limited data sets, where estimates of the error in the mean cannot be obtained. When reliable estimates of the mean and standard deviation of growth responses of organisms become available, it will be possible to predict both the extent of growth in a particular situation and confidence limits for that prediction, to make objective risk assessments and to 'fine-tune' predictions for specific 'acceptable risk levels'. Without reliable estimates the safest alternative is to adopt a 'worst case scenario' to accommodate inconsistent growth responses.

APPENDIX

There have been a number of models proposed for use in predictive microbiology. The following discussion briefly traces the development of the 'Schoolfield' model and the 'square root' model.

Arrhenius-type models

The model of Schoolfield *et al.* (1981) is simply a reparameterization, without change to the model theory, to give better parameter estimation properties to the model proposed by Sharpe & DeMichele (1977). Both models assume that growth rate is governed by a single rate-limiting enzyme-catalysed reaction, the rate of which may be described by the Arrhenius equation with additional thermodynamic terms to describe the rate-modifying effects of high and low temperature inactivation of the rate-limiting enzyme. Both models are a synthesis of the equations proposed by Hultin (1955) and Johnson & Lewin (1946). Adair *et al.* (1989) reparameterized the model of Schoolfield *et al.* (1981) and expressed it with ln(*time*), rather than *rate*, as the dependent variable. These models with a description of their parameters are presented below.

The model of Johnson & Lewin (1946)

$$\text{Rate} = \frac{c\,.\,T\,.\,\exp(-\Delta H^{+}/RT)}{1 + \exp(\Delta S/R)\,.\,\exp(-\Delta H/RT)} \qquad \text{(A1)}$$

where c = a constant; T = temperature in degrees Kelvin; R = the gas constant; ΔH^{+} = the heat (enthalpy) of activation of the growth rate-controlling reaction; ΔS = entropy of denaturation of the rate-controlling enzyme; ΔH = enthalpy of denaturation of the rate-controlling enyzme.

In this model the numerator

$$c\,.\,T\,.\,\exp(-\Delta H^{+}/RT)$$

has the form of an Arrhenius relationship, as modified by Eyring (1935), and is used to describe the kinetics of the growth rate-governing reaction, which is assumed to be enzyme-catalysed. To account for the deviation of observed growth response from that predicted by the above numerator, the model proposes that the growth rate-governing enzyme becomes inactivated at high temperatures in a manner also described by a unique Arrhenius model

$$[\text{enzyme}_{\text{active}}] = [\text{enzyme}_{\text{total}}]\,.\,\exp(\Delta S/R)\,.\,\exp(-\Delta H/RT)$$

i.e. this function models the temperature-dependent transition of the growth rate-controlling enzyme between an active and an inactive state. Thus, the overall growth rate is determined by the kinetics of the reaction catalysed by the growth rate-governing enzyme and also the proportion of that enzyme population in an active state. These two temperature-dependent processes are mathematically 'superimposed' to yield the full model. For temperatures at which the rate of reaction from the active to inactive state is close to zero, the denominator approximates to unity, and the growth rate is predicted by the numerator. As the rate of transition to the inactive form of the growth rate-governing enzyme increases, i.e. $\exp(\Delta S/R)\,.\,\exp(-\Delta/RT)$ has values greater than zero, the denominator takes values greater than unity. Thus the prediction of the above numerator is 'corrected' for the effects of enzyme inactivation caused by high temperatures.

The model of Hultin (1955) for the effect of temperature on enzyme activity

$$\text{Rate} = \frac{xkT}{h}\,.\,\frac{\exp \Delta S^{+}/R\,.\,\exp\,(-\Delta H^{+}/RT)}{1 + K} \qquad \text{(A2)}$$

where T, R and $-\Delta H^{+}$ have the same meaning as in equation A1; k = the Boltzmann constant; h = Planck's constant; x = the transmission constant; ΔS^{+} = the entropy of the enzyme-catalysed reaction; $K = \exp\{(-\Delta H/R)\,.\,[(1/T) - (1/T_{1/2})]\}$; $T_{1/2} = \Delta H/\Delta S$, the temperature at which half of the population of the enzyme is active and half has been inactivated by low temperature; ΔH = the enthalpy of (low temperature) denaturation of the enzyme.

This model was developed to describe the effect of temperature on enzyme activity rather than microbial growth rate. Nonetheless, with the parameter K shown in full, this model is mathematically equivalent to the Johnson & Lewin (1946) model. Hultin introduced the term $T_{1/2}$, which can be evaluated by graphical methods, to facilitate determination of the parameters ΔH and $-\Delta H^{+}$.

The model of Sharpe & DeMichele (1977)

$$\text{Rate} = \frac{T \exp[(\phi - \Delta H_A^{+}/T)/R]}{1 + \exp[(\Delta S_L - \Delta H_L/T)/R] + \exp[(\Delta S_H - \Delta H_H/T)/R]} \qquad \text{(A3)}$$

where T, R have the same meaning as previously; ϕ = a constant, similar to that in the Johnson & Lewin (1946) model, encompassing the entropy of activation of the rate-controlling enzyme, enzyme concentration, and Planck's and Boltzmann's constants; $\Delta H_A^\ddagger$ = the heat (enthalpy) of activation of the growth rate-controlling reaction; ΔS_L = entropy of low temperature denaturation of the rate-controlling enzyme; ΔH_L = enthalpy of low temperature denaturation of the rate-controlling enzyme; ΔS_H = entropy of high temperature denaturation of the rate-controlling enzyme; ΔH_H = enthalpy of high temperature denaturation of the rate-controlling enzyme.

Sharpe & DeMichele's model also assumes a growth rate-controlling enzyme. The rate of the reaction catalysed by this enzyme, when all of the enzyme population is in the active state, is modelled by the numerator. The denominator includes terms which model the transition between the active and inactive form of the enzyme for both high and low temperature inactivations.

The model of Schoolfield et al. *(1981)*

Rate =

$$\frac{\rho(25°\mathrm{C})\frac{T}{298}\exp\left[\frac{\Delta H_A^\ddagger}{R}\left(\frac{1}{298}-\frac{1}{T}\right)\right]}{1+\exp\left[\frac{\Delta H_L}{R}\left(\frac{1}{T_{1/2_L}}-\frac{1}{T}\right)\right]+\exp\left[\frac{\Delta H_H}{R}\left(\frac{1}{T_{1/2_H}}-\frac{1}{T}\right)\right]} \qquad (A4)$$

where all the parameters have the same meaning as those of the Sharpe & DeMichele (1977) model, but with three new parameters defined as follows: $\rho(25°\mathrm{C}) = 298.\exp(\phi - \Delta H_A^\ddagger/298)/R$ and is analogous to ϕ in equation A3; $T_{1/2_L} = \Delta H_L/\Delta S_L$ and is the temperature at which half of the population of the rate-controlling enzyme is active and the other half has been inactivated by low temperature; $T_{1/2_H} = \Delta H_H/\Delta S_H$ and is the temperature at which half of the population of the rate-controlling enzyme is active and the other half has been inactivated by high temperature.

The latter two parameters are analogous to Hultin's $T_{1/2}$ and also represent the low and high temperatures respectively at which the equilibrium constants for the activation/inactivation reaction for the rate-controlling enzyme are 1·0. They are introduced because they permit graphical determination of parameter values.

Adair *et al.* (1989) developed an alternative form of the Schoolfield *et al.* (1981) model which has improved numerical stability and increased speed of convergence when data are fitted to the model by the NLIN non-linear regression procedure of SAS (Statistical Analysis System). In this model ln *time* is the dependent variable. The form of the model is

$$\ln(K) = A + (B/T) - \ln T + \ln\{1 + \exp[F + (D/T)] + \exp[G + (H/T)]\} \qquad (A5)$$

where K = lag or generation time; $A = \ln 298 - \{[\Delta H_A^\ddagger/(298.R)] - \ln \rho(25°\mathrm{C})\}$; $B = -\Delta H_A^\ddagger/R$; $D = -\Delta H_L/R$; $F = -\Delta H_L/(T_{1/2_L}.R)$; $G = -\Delta H_H/(T_{1/2_H} R)$; $H = -\Delta H_H/R$; and where R, T, $\Delta H_A^\ddagger$, $-\Delta H_H$, $-\Delta H_L$, $T_{1/2_H}$, $T_{1/2_L}$ and $\ln \rho(25°\mathrm{C})$ are as defined above. For data in the suboptimal temperature range the model is simplified by the deletion of the term: $\exp[G + (H/T)]$. If the full expression for $\rho(25°\mathrm{C})$ is substituted into the four-parameter form of equation A5, it simplifies to the form of equations A1 and A2.

Bělehrádek-type models

The square root models of Ratkowsky *et al.* (1982, 1983) to describe the effect of temperature on bacterial growth rate were developed from the model of Ohta & Hirahara (1977) which describes the effects of temperature on nucleotide degradation. Subsequently Ross (1986) showed that the simple square root model was a special case of Bělehrádek's temperature function in which the exponent has the value two. This relationship was developed by Bělehrádek (1926a, b; 1935). These models and a description of their parameters are shown below.

The model of Ratkowsky et al. *(1982)*

$$\sqrt{k} = b(T - T_{min}) \qquad (A6)$$

where k = rate of growth; T = temperature in degrees K; T_{min} = a theoretical lower temperature limit for growth at and below which the predicted rate of growth is zero; b = a parameter; the regression coefficient of the square root of rate versus sub-optimal temperature.

The model of Ratkowsky et al. *(1983)*

$$\sqrt{k} = b(T - T_{min})\{1 - \exp[c(T - T_{max})]\} \qquad (A7)$$

where b, T, and T_{min} have the same meaning as in equation A6; T_{max} = the upper temperature limit at and beyond which the predicted rate of growth is zero; c = an additional parameter to enable the model to fit the data at temperatures near and above the optimal temperature for growth.

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