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New modified Square Root and Schoolfield models for predicting bacterial growth rate as a function of temperature

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SUMMARY

A new modified Square Root model and two new modified Schoolfield models were evaluated for their ability to predict the growth rate of *Yersinia enterocolitica* as a function of temperature. The new Square Root model fits the data better than both the original Square Root model and the Zwietering Square Root model. Both new Schoolfield models, a six- and a four-parameter equation, fit the data better than the original Schoolfield model. The new four-parameter Schoolfield model was developed by removing the term describing low temperature inactivation from the new six-parameter Schoolfield model. Inclusion of the two extra parameters in the new six-parameter Schoolfield model (F = 318) did not significantly improve the fit compared to the new four-parameter Schoolfield model (F = 488).

INTRODUCTION

The Square Root and Schoolfield models predict bacterial growth rate or lag time as a function of temperature. These models have typically been applied with either the square root or the natural logarithm of growth rate as the dependent variable [4]. Alber and Schaffner [1] discussed the statistical consequences of these data transformations. Homogeneity of variance is an important stochastic assumption for regression analysis. Data transformation influences variance homogeneity by disproportionally altering the variance associated with each growth rate. The variances of the transformed growth rates should be examined for homogeneity [1,4].

The experimental procedure frequently employed for determining growth rates at different temperatures results in nonhomogeneous variances. Growth rates are typically calculated from a series of concentration measurements at intervals throughout the entire exponential phase of growth. At favorable growth temperatures the organism reaches the stationary phase rapidly and concentration measurements may be made at short time intervals over a period of hours. At suboptimal temperatures it may take days or weeks for the organism to reach stationary phase. Measurements are usually taken throughout this longer log phase at large time intervals. This difference in time range results in growth rate variances which increase as the magnitude of growth rate increases [1].

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Data transformation can convert growth rate to a scale in which the variances are homogeneous. It has been shown that the natural logarithm is an appropriate transformation for growth rate data of *Yersinia enterocolitica* in brain heart infusion (BHI) broth [1]. This data set is used in this paper. Therefore all of the models compared here are applied with a natural logarithm transformation.

Square Root models

The Square Root model was originally proposed by Ratkowsky et al. [3]. A natural logarithm transformation of the Square Root model is given as:

$$\ln(k) = 2\ln(b(T - T_{\min})\{1 - \exp[c(T - T_{\max})]\}) \qquad (1)$$

k = growth rate (time^{-1})
b = regression coefficient (K^{-1} $\text{time}^{-0.5}$)
T = temperature (K)
$T_{\min}$ = notional minimum growth temperature (K)
c = regression coefficient (K^{-1})
$T_{\max}$ = notional maximum growth temperature (K)

In Eqn 1 positive growth rates are predicted at temperatures above $T_{\max}$. Zwietering et al. [7] proposed a modification of the Square Root model which results in predictions of negative growth rates at temperatures above $T_{\max}$. The Zwietering modified Square Root model with a natural logarithm transformation is given as:

$$\ln(k) = \ln([b(T - T_{\min})]^2\{1 - \exp[c(T - T_{\max})]\}) \qquad (2)$$

A new Square Root model was developed from Eqn 2 and is given as:

$$\ln(k) = 2\ln[\ln([b(T - T_{min})]^2\{1 - \exp[c(T - T_{max})]\})] \quad (3)$$

None of the parameters in Eqn 3 retain the same biological interpretation as in Eqns 1 and 2. Eqn 3 with k as the dependent variable is given as

$$k = [\ln([b(T - T_{min})]^2\{1 - \exp[c(T - T_{max})]\})]^2 \quad (4)$$

Schoolfield models

The Schoolfield model was originally proposed by Sharpe and DeMichele [6] and subsequently modified by Schoolfield et al. [5]. The Schoolfield equation with a natural logarithm transformation is given as:

$$\ln(k) = \ln\left[\frac{\rho(25\ ^\circ\mathrm{C})\frac{T}{298}\exp\left[\frac{\Delta H_A^{\ne}}{R}\left(\frac{1}{298} - \frac{1}{T}\right)\right]}{1 + \exp\left[\frac{\Delta H_L}{R}\left(\frac{1}{T_{\frac{1}{2}L}} - \frac{1}{T}\right)\right] + \exp\left[\frac{\Delta H_H}{R}\left(\frac{1}{T_{\frac{1}{2}H}} - \frac{1}{T}\right)\right]}\right] \quad (5)$$

k = growth rate (time^{-1})
$\rho(25\ ^\circ\mathrm{C})$ = growth rate at 25 °C (time^{-1})
T = temperature (K)
R = universal gas constant (8.314 J K^{-1} mol^{-1})
$\Delta H_A^{\ne}$ = enthalpy of activation of the reaction catalyzed by the rate controlling enzyme (J mol^{-1})
ΔH_L = change in enthalpy associated with low temperature inactivation of the enzyme (J mol^{-1})
$T_{\frac{1}{2}L}$ = temperature at which the enzyme is 50% inactive because of low temperature (K)
ΔH_H = change in enthalpy associated with high temperature inactivation of the enzyme (J mol^{-1})
$T_{\frac{1}{2}H}$ = temperature at which the enzyme is 50% inactive because of high temperature (K)

A new six-parameter Schoolfield model was developed and is given as:

$$\ln(k) = 2\ln\left(\ln\left[\frac{\rho(25\ ^\circ\mathrm{C})\frac{T}{298}\exp\left[\frac{\Delta H_A^{\ne}}{R}\left(\frac{1}{298} - \frac{1}{T}\right)\right]}{1 + \exp\left[\frac{\Delta H_L}{R}\left(\frac{1}{T_{\frac{1}{2}L}} - \frac{1}{T}\right)\right] + \exp\left[\frac{\Delta H_H}{R}\left(\frac{1}{T_{\frac{1}{2}H}} - \frac{1}{T}\right)\right]}\right]\right) \quad (6)$$

None of the parameters in Eqn 6 retain the same biological interpretation as in Eqn 5. Eqn 6 with no transformation is given as:

$$k = \left[\ln\frac{\rho(25\ ^\circ\mathrm{C})\frac{T}{298}\exp\left[\frac{\Delta H_A^{\ne}}{R}\left(\frac{1}{298} - \frac{1}{T}\right)\right]}{1 + \exp\left[\frac{\Delta H_L}{R}\left(\frac{1}{T_{\frac{1}{2}L}} - \frac{1}{T}\right)\right] + \exp\left[\frac{\Delta H_H}{R}\left(\frac{1}{T_{\frac{1}{2}H}} - \frac{1}{T}\right)\right]}\right]^2 \quad (7)$$

Eqn 6 with $\sqrt{k}$ as the dependent variable is given as:

$$\sqrt{k} = \ln\left[\frac{\rho(25\ ^\circ\mathrm{C})\frac{T}{298}\exp\left[\frac{\Delta H_A^{\ne}}{R}\left(\frac{1}{298} - \frac{1}{T}\right)\right]}{1 + \exp\left[\frac{\Delta H_L}{R}\left(\frac{1}{T_{\frac{1}{2}L}} - \frac{1}{T}\right)\right] + \exp\left[\frac{\Delta H_H}{R}\left(\frac{1}{T_{\frac{1}{2}H}} - \frac{1}{T}\right)\right]}\right] \quad (8)$$

A second new Schoolfield model was developed by removing the term which describes low temperature inactivation from Eqn 6. This new four-parameter modified Schoolfield model is given as:

$$\ln(k) = 2\ln\left(\ln\left[\frac{\rho(25\ ^\circ\mathrm{C})\frac{T}{298}\exp\left[\frac{\Delta H_A^{\ne}}{R}\left(\frac{1}{298} - \frac{1}{T}\right)\right]}{1 + \exp\left[\frac{\Delta H_H}{R}\left(\frac{1}{T_{\frac{1}{2}H}} - \frac{1}{T}\right)\right]}\right]\right) \quad (9)$$

The three new models (Eqns 3, 6 and 9) were evaluated for their ability to predict the growth rate of *Y. enterocolitica* in BHI broth. The new Square Root model was compared with the original Square Root model and with the Square Root model developed by Zwietering et al. [7]. The new six-parameter and the new four-parameter Schoolfield models were compared with the original Schoolfield model.

MATERIALS AND METHODS

All model parameter estimates were determined with Tablecurve 3.01 (Jandel Scientific, Corte Madera, CA), which uses the Levenberg–Marquardt algorithm.

Initial parameter estimates for the original Square Root model (Eqn 1) and the Zwietering modified Square Root model (Eqn 2) were determined from the procedure given by Ratkowsky et al. [3]. The final parameter estimates from the Zwietering modified Square Root model were used as initial parameter estimates for the untransformed new Square Root model (Eqn 4). The resulting parameter estimates were then used as initial parameter estimates for the natural logarithm transformed new Square Root model (Eqn 3).

Starting parameter values for the original Schoolfield model (Eqn 5) were calculated by the method given by Schoolfield et al. [5]. The final parameter estimates from the original Schoolfield model were used as initial parameter estimates for the square root transformed new six-parameter Schoolfield model (Eqn 7). The resulting final parameter estimates were in turn used as initial parameter estimates

TABLE 1

Square Root models

Model	Eqn number	SSE	Parameter estimates	t-value
Square Root	1	0.397	b = 0.0316	30.6
			T_{min} = 267	651
			c = 0.627	4.72
			T_{max} = 316	1954
			b = 0.0320	30.9
Zwietering Square Root	2	0.356	T_{min} = 267	684
			c = 0.413	4.57
			T_{max} = 315	4056
			b = 0.0209	33.6
New Square Root	3	0.294	T_{min} = 221	134
			c = 0.744	4.18
			T_{max} = 316	1763

All models fitted with a natural logarithm transformation. SSE = sum of squared error on the logarithmic scale.

for both the new six-parameter (Eqn 6) and the new four-parameter (Eqn 9) Schoolfield models.

RESULTS AND DISCUSSION

Square Root models

Regression results of the Square Root models are given in Table 1. The Zwietering Square Root model (Eqn 2) has a 9.6% lower sum of squared error (SSE) when compared to the original Square Root model (Eqn 1). The new Square Root model (Eqn 3) has a 17.4% smaller SSE than the Zwietering Square Root model and a 25.9% smaller SSE than the original Square Root model.

Fig. 1 shows that above 306 K the original Square Root model (A) generally overpredicts growth rates, whereas both the Zwietering (B) and the new Square Root (C) models generally predict growth rates which are smaller and closer to the observed values. *Y. enterocolitica* loses motility above 303 K [2]. This may have depressed the growth rate at temperature greater than 303 K and may have resulted in the original Square Root model overpredicting growth rate in this temperature range.

The new Square Root model contains several singularities which resulted in computational difficulties during regression. A singularity occurs when a mathematical expression is undefined. Examples include division by zero and the logarithm of a number less than or equal to zero. If a singularity is encountered or closely approached during regression it prevents the algorithm from converging. The precision of the software will control the sensitivity of the algorithm to the singularities. The likelihood of encountering or closely approaching a singularity is dependent on the data.

Singularities occur in the untransformed new Square Root model (Eqn 4) when T_{min} or T_{max} equals T and when b or c equals zero. Any of these parameter values will result in $[b(T - T_{min})]^2\{1 - \exp[c(T - T_{max})]\}$ equaling zero, and $\ln([b(T - T_{min})]^2\{1 - \exp[c(T - T_{max})]\})$ being undefined. Due to the natural logarithm transformation, the same singularities occur in Eqns 1 and 2. An additional singularity is introduced into the new Square Root model by the natural logarithm transformation. When $[b(T - T_{min})]^2\{1 - \exp[c(T - T_{max})]\}$ is between zero and one the natural logarithm of this expression is a negative value. When $[b(T - T_{min})]^2$ $\{1 - \exp[c(T - T_{max})]\}$ is equal to one the natural logarithm of this expression is zero. In either case $\ln[\ln([b(T - T_{min})]^2\{1 - \exp[c(T - T_{max})]\})]$ is undefined (Ratkowsky, personal communication).

The initial parameter values influence the likelihood of encountering singularities in the new Square Root model. When the final parameter estimates from the Zwietering Square Root model (Eqn 2) were used as the initial parameters estimates for the new Square Root model (Eqn 3), a singularity was encountered and the algorithm fails to converge. This combination of initial parameter estimates resulted in $[b(T - T_{min})]^2\{1 - \exp[c(T - T_{max})]\}$ approaching 1 at observed T values of 296 and 301 K. When this expression equals 1 the algorithm encounters a singularity and terminates before convergence.

The singularities in the new Square Root model (Eqn 3) may be avoided by a judicious choice of starting parameter

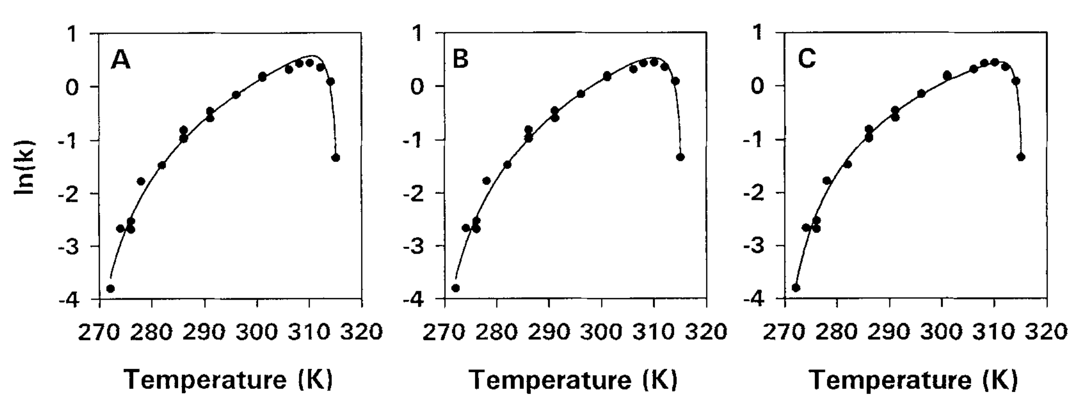

Fig. 1. Square Root models: (A) original Square Root model (Eqn 1); (B) Zwietering Square Root model (Eqn 2); (C) new Square Root model (Eqn 3).

estimates. Regression with the untransformed new Square Root model (Eqn 4) converged properly, because the model does not contain the additional singularity introduced by the natural logarithm transformation. By using the final parameter estimates from Eqn 4 as initial parameter estimates for Eqn 3 the singularities were avoided and the algorithm converged properly. Eqns 3 and 4 are different transformations of the same model. Although their parameter values are not equal, they are similar.

Schoolfield models

A comparison of the Schoolfield models is given in Fig. 2. As with the Square Root models, the modification of the Schoolfield model improved the fit of the data. The SSE for the new six-parameter Schoolfield model is 33% lower than the SSE for the original Schoolfield model.

The new six-parameter Schoolfield model (Eqn 6) contains singularities associated with the natural logarithm which parallel those in the new Square Root model. These singularities occur when a combination of parameter values result in the argument of either of the two logarithms equaling zero or a negative number. For the same reason that final parameter estimates from the Zwietering Square Root model (Eqn 2) cannot be used as initial parameter estimates for the logarithm transformed new Square Root model (Eqn 3), final parameter estimates from the Schoolfield model (Eqn 5) cannot be used as initial parameter estimates for the natural logarithm transformed new six-parameter Schoolfield model (Eqn 6).

The regression algorithm failed to converge when final parameter estimates from Eqn 5 were used as initial parameter estimates for Eqn 7. However, the regression algorithm converged successfully when final parameter estimates from Eqn 5 were used as initial parameter estimates for the square root transformed new six-parameter Schoolfield model (Eqn 8). When the final parameter estimates from Eqn 8 were used as initial parameter estimates for Eqn 6 the singularities were circumvented allowing the algorithm to converge successfully.

The new six-parameter Schoolfield model may be overparameterized for the data set. Table 2 shows that four of the parameters do not contribute significantly to the model predictions ($\alpha = 0.05$). Removing the term which describes low temperature inactivation produced a new four-parameter Schoolfield model (Eqn 9) in which all of the parameters contribute significantly to the model predictions. The improvement of the new six-parameter model over the four-parameter model seen in Fig. 2 is not significant (F = 318, F = 488, respectively).

CONCLUSIONS

All of the new models (Eqns 3, 6 and 9) fit the data better than the original Square Root model, the Zwietering Square Root model, and the original Schoolfield model.

TABLE 2

Schoolfield models

Model	Eqn number	SSE	Parameters	*t*-value
Schoolfield	5	0.412	ρ25 °C = 0.883	16.3
			$\Delta H^{\neq}_{A}$ = 8.51 × 10^3	6.68
			ΔH_{L} = −4.25 × 10^4	−5.56
			$T_{\frac{1}{2}L}$ = 279	162
			ΔH_{H} = 4.11 × 10^5	4.88
			$T_{\frac{1}{2}H}$ = 314	1629
New six-parameter Schoolfield	6	0.275	ρ25 °C = 1.68 × 10^3	0.217*
			$\Delta H^{\neq}_{A}$ = −5.94 × 10^4	−0.963*
			ΔH_{L} = −6.40 × 10^4	−1.04*
			$T_{\frac{1}{2}L}$ = 317	60.6
			ΔH_{H} = 4.35 × 10^5	0.554*
			$T_{\frac{1}{2}H}$ = 315	242
New four-parameter Schoolfield	9	0.337	ρ25 °C = 2.64	51.6
			$\Delta H^{\neq}_{A}$ = 4.48 × 10^4	31.3
			ΔH_{H} = 2.16 × 10^5	5.13
			$T_{\frac{1}{2}H}$ = 315	3054

* Parameter does not significantly contribute to model predictions ($\alpha = 0.05$).

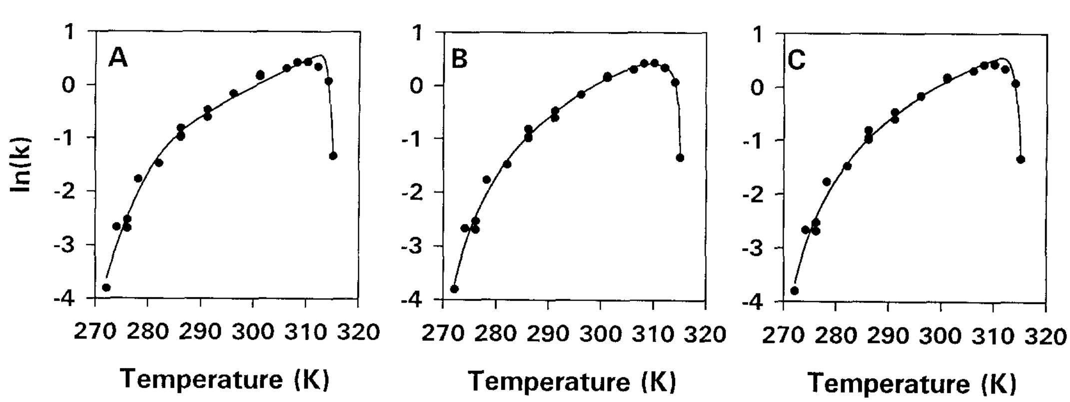

Fig. 2. Schoolfield models: (A) original Schoolfield model (Eqn 5); (B) new six-parameter Schoolfield model (Eqn 6); (c) new four-parameter Schoolfield model (Eqn 9).

The new four-parameter Schoolfield model has a better fit than the original six-parameter Schoolfield model, is more parsimonious and has tighter parameter confidence intervals, as indicated by larger parameter t-values.

One of the goals in predictive microbiology is to develop models which fit microbiological data as precisely as possible, without violating any statistical or biological principles. The new models presented in this paper have an improved fit to our data set when compared to the original unmodified models. A future objective is to determine if these new models are suitable for other organisms.

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REFERENCES

1 Alber, S.A. and D.W. Schaffner. 1992. Evaluation of data transformations used with the square root and Schoolfield models for predicting bacterial growth rate. Appl. Environ. Microbiol. 58: 3337–3342.

2 Krieg, N.R. (ed.) 1984. Bergey's Manual of Systematic Bacteriology, Vol. 1, p. 498. The Williams & Wilkins Co., Baltimore.

3 Ratkowsky, D.A., R.K. Lowry, T.A. McMeekin, A.N. Stokes and R.E. Chandler. 1983. Model for bacterial culture growth rate throughout the entire biokinetic temperature range. J. Bacteriol. 154: 1222–1226.

4 Ratkowsky, D.A., T. Ross, T.A. McMeekin and J. Olley. 1991. Comparison of Arrhenius-type and Bělehrádek-type models for prediction of bacterial growth in foods. J. Appl. Bacteriol. 71: 452–459.

5 Schoolfield, R.M., P.J.H. Sharpe and C.E. Magnuson. 1981. Non-linear regression of biological temperature-dependent rate models based on absolute reaction-rate theory. J. Theor. Biol. 88: 719–731.

6 Sharpe, P.J.H. and D.W. DeMichele. 1977. Reaction kinetics of poikilotherm development. J. Theor. Biol. 64: 649–670.

7 Zwietering, M.H., J.T. de Koos, B.E. Hasenack, J.C. de Wit and K. van't Riet. 1991. Modeling of bacterial growth as a function of temperature. Appl. Environ. Microbiol. 57: 1094–1101.