JOURNAL OF BACTERIOLOGY, June 1983, p. 1222–1226
0021-9193/83/061222-05$02.00/0

Vol. 154, No. 3

Model for Bacterial Culture Growth Rate Throughout the Entire Biokinetic Temperature Range

D. A. RATKOWSKY,[1] R. K. LOWRY,[1] T. A. McMEEKIN,[2]* A. N. STOKES,[3] AND R. E. CHANDLER[2]

Department of Agricultural Science, University of Tasmania,[2] and Division of Mathematics and Statistics, Commonwealth Scientific and Industrial Research Organization,[1] Hobart, Tasmania, Australia 7000, and Division of Mathematics and Statistics, Commonwealth Scientific and Industrial Research Organization, Melbourne, Victoria, Australia 3168[3]

Received 23 December 1982/Accepted 4 March 1983

The "square-root" relationship proposed by Ratkowsky et al. (J. Bacteriol. 149:1–5, 1982) for modeling the growth rate of bacteria below the optimum growth temperature was extended to cover the full biokinetic temperature range. Two of the four parameters of this new nonlinear regression model represent minimum and maximum temperature bounds, respectively, for the predicted growth of the culture. The new model is easy to fit and has other desirable statistical properties. For example, the least-squares estimators of the parameters of the model were almost unbiased and normally distributed. The model applied without exception to all bacterial cultures for which we were able to obtain data. Results for 30 strains are reported.

Althouth the Arrhenius law for chemical reactions has often been applied by microbiologists to bacterial growth, Ratkowsky et al. (6) showed that it fits data poorly. Graphs of the logarithm of the growth rate constant against the reciprocal absolute temperature (socalled Arrhenius plots) are curves rather than straight lines. In place of the Arrhenius law, Ratkowsky et al. (6) proposed a linear relationship between the square root of the growth rate constant (r) and the absolute temperature (T) in degrees Kelvin:

$$\sqrt{r} = b(T - T_0) \quad (1)$$

where b is a regression coefficient and T_0 is a notional temperature which is an intrinsic property of the organism. This relationship was found to apply to data for 43 strains of bacteria for temperatures ranging from the minimum temperature at which growth is observed to just below the optimum temperature (T_{opt}), at which maximum growth occurs. At higher temperatures, equation 1 ceases to model growth adequately owing to the inactivation or denaturation of proteins or to other factors. We now propose an extension of equation 1 which is capable of describing bacterial growth throughout the entire temperature range. The new empirical nonlinear regression model is

$$\sqrt{r} = b(T - T_{min})\{1 - \exp[c(T - T_{max})]\} \quad (2)$$

where T_{min} and T_{max} are the minimum and maximum temperatures, respectively, at which the rate of growth is zero. The parameter b, as in equation 1, is the regression coefficient of the square root of growth rate constant versus degrees Kelvin for temperatures below the optimal temperature, whereas c is an additional parameter to enable the model to fit the data for temperatures above the optimal temperature. The temperature T_{min} corresponds to T_0 in equation 1. It may be a conceptual temperature of no metabolic significance for psychrophiles, psychrotrophs, and mesophiles, but it could be a realizable temperature condition for thermophiles when T_{min} exceeds the freezing point of water. When T is much lower than T_{max}, the contribution of the term in braces is negligible, and equation 2 reduces to equation 1. As T increases to approach T_{max}, the term becomes increasingly more important until eventually it dominates, and the growth rate falls as T exceeds T_{opt}, reaching zero when $T = T_{max}$.

MATERIALS AND METHODS

Strains and growth conditions. The organisms used are listed in Tables 1 through 3. The first 12 strains listed in Table 1 were studied by one of us (R.E.C.) and were obtained from samples of chicken neck skin which were spoiled at various temperatures. The effect of temperature on the growth of these isolates was examined in a temperature gradient incubator (Toyo Kagaku Sangyo Co., Ltd., Tokyo, Japan). Growth was examined over a temperature range of 7 to 43°C at approximately 1°C intervals. The growth medium (nutrient broth, Oxoid, London) was inoculated with 1.0

TABLE 1. Predicted minimum, maximum, and optimum growth temperatures for 16 bacterial cultures

Organism	Strain no.	Predicted temp range (K)		
		T_{min}	T_{opt}	T_{max}
Acinetobacter sp.	2.55	271	301	309
Acinetobacter sp.	4.41	271	303	311
Acinetobacter sp.	6.12	268	302	311
Acinetobacter sp.	3.25	274	305	315
Pseudomonas sp. group I	6.4	267	299	304
Pseudomonas sp. group I	4.54	272	304	310
Pseudomonas sp. group II	2.3	266	300	310
Pseudomonas sp. group II	5.16	269	302	313
Flavobacterium (*Cytophaga*) sp.	6.32	270	301	310
Flavobacterium (*Cytophaga*) sp.	2.4	269	302	310
Aeromonas sp.	4.29	277	309	320
Moraxella sp.	4.16	272	303	314
Bacillus stearothermophilus	238	303	331	341
Proteus morganii	M68	272	310	318
Alteromonas sp.	CLD38	267	299	309
Pseudomonas sp. group I	16L16	266	302	310

ml of each culture grown in nutrient broth for 19 h at 25°C. Growth at each temperature was determined by optical density measurements with a nephelometer (Corning Unigalvo, Essex). The growth rate was calculated at each temperature as the reciprocal of the time required for the culture to reach a turbidity level of 35%. The growth rate determined in this manner may be used as a constant if the same initial amount of inoculum and the same final turbidity are used. The time lag between the initial and final states will then be a multiple of the mean doubling time, or instantaneous generation time. The multiplicative factor is absorbed into parameter b in equation 2 and does not affect the values of the other three parameters. The *Bacillus stearothermophilus* strain (Table 1) was isolated from a fish-eucalyptus bark compost, and its temperature behavior was determined as described above by using a range of 30 to 80°C. The *Proteus morganii* strain was obtained from the University of Queensland, Australia. The temperature range was 19 to 42°C. The last two strains listed in Table 1 were examined by Andrew Ball (B.Sc.[Hons] thesis, University of Tasmania, Hobart, Tasmania, Australia, 1980) using techniques already described (6). In addition to our original growth curves (Table 1), we also used the previously published data of Mohr and Krawiec (4) for 12 strains of bacteria (Table 2) and the previously published data of Reichardt and Morita (7) for a psychrotrophic strain of *Cytophaga johnsonae* (Table 3).

Statistical methods. As equation 2 is nonlinear in its parameters, initial parameter estimates are required for the method of least-squares regression. Estimates of b and T_{min} may be determined from the straight-line portion of the graph of $\sqrt{r}$ versus T, for which equation 1 provides a good approximation. The exact number of temperature used is not critical. After obtaining estimates for b and T_{min}, equation 2 may be rearranged to give

$$\log\left[1 - \frac{\sqrt{r}}{b(T - T_{min})}\right] = c(T - T_{max}) \quad (3)$$

TABLE 2. Predicted minimum, maximum, and optimum growth temperatures for 12 bacterial cultures[a]

Organism	Strain no.	Predicted temp range (K)		
		T_{min}	T_{opt}	T_{max}
Vibrio psychroerythrus	ATCC 27364	276	286	293
Vibrio marinus	ATCC 15381	276	288	294
Vibrio marinus	ATCC 15382	277	297	303
Serratia marcescens		278	308	314
Pseudomonas fluorescens		279	312	320
Escherichia coli		276	314	322
Bacillus subtilis		284	312	326
Bacillus megaterium		280	314	325
Pseudomonas aeruginosa		272	312	320
Bacillus stearothermophilus		308	337	349
Bacillus coagulans		290	325	338
Thermus aquaticus		306	344	357

[a] Data from Mohr and Krawiec (4).

TABLE 3. Predicted minimum, maximum, and optimum growth temperatures for *C. johnsonae* C21[a]

Preincubation temp (°C)	Predicted temp range (K)		
	T_{min}	T_{opt}	T_{max}
10	265	297	307
23	265	301	308

[a] Data from Reichardt and Morita (7).

The left-hand side of equation 3 can be numerically evaluated by any data point. From two data points in the high-temperature region, estimates of c and T_{max} may be obtained. The procedure is readily automated to provide initial estimates with a routine based on the Gauss-Newton algorithm (3) for finding the least-squares estimates. The properties of the least-squares estimators were studied by using the curvature measures of nonlinearity described by Bates and Watts (1) and the bias measure of Box (2). The meaning and use of these measures are described elsewhere (5).

RESULTS AND DISCUSSION

The results for the fit of equation 2 to the original data for 16 cultures are shown in Table 1 and Fig. 1. The results for 12 additional cultures with the data of Mohr and Krawiec (4) are given in Table 2 and Fig. 2, and the results for a strain of *C. johnsonae* are given in Table 3 and Fig. 3.

The T_{opt} values varied by almost 60 K from the psychrophilic to the thermophilic range. In

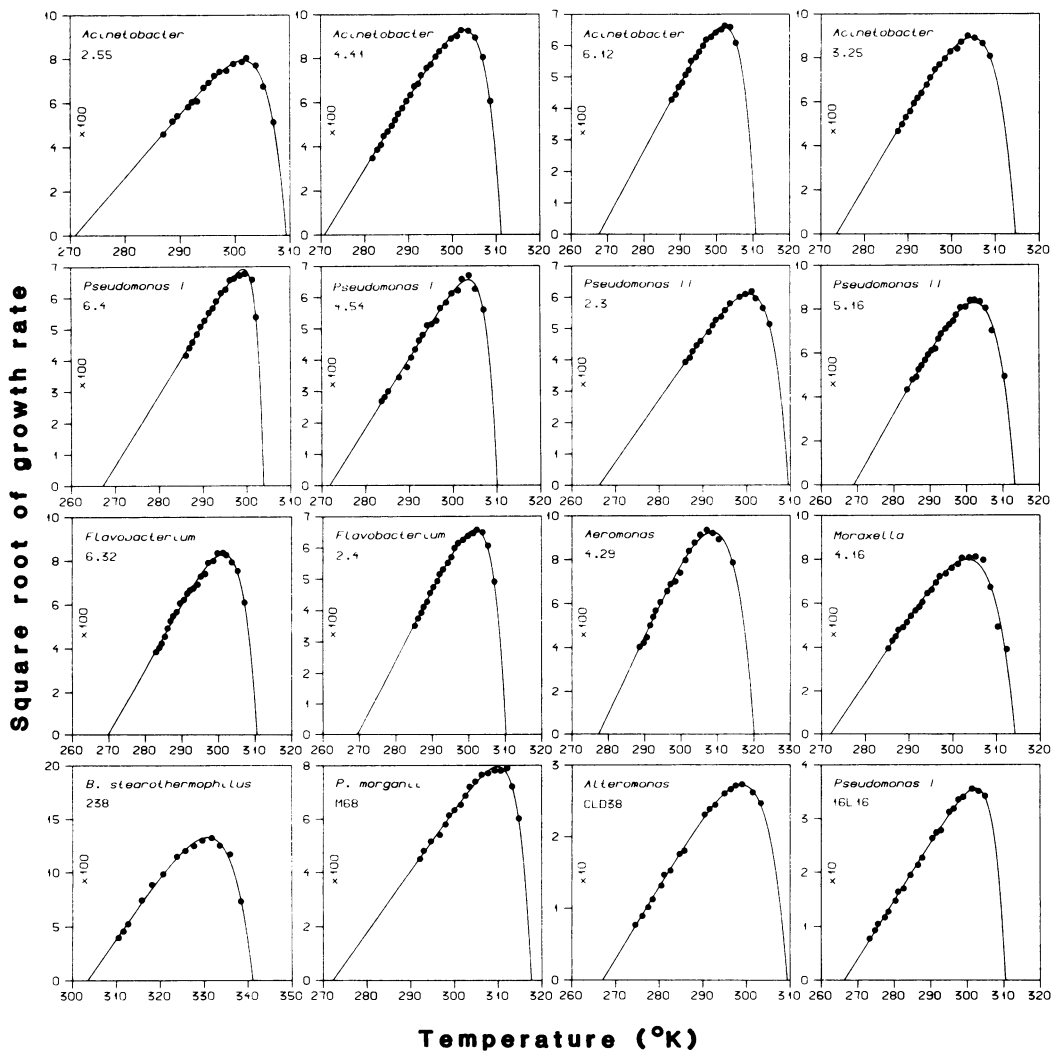

FIG. 1. Data and fitted lines of equation 2 for 16 bacterial cultures.

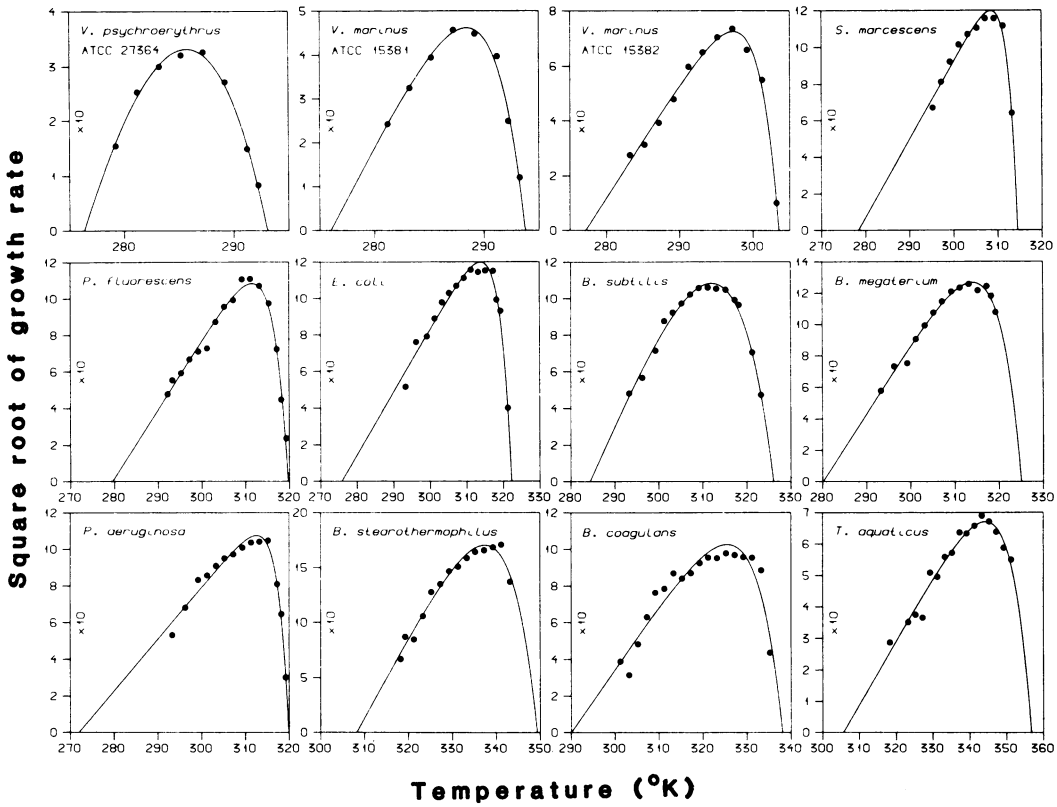

FIG. 2. Data and fitted lines of equation 2 for 12 bacterial cultures studied by Mohr and Krawiec (4).

all cases, the Gauss-Newton algorithm converged quickly to the least-squares estimates of the parameters using the initial estimates obtained as described above. The 30 curves (Fig. 1 through 3) showed the close fit of equation 2 to the data and the lack of any overall systematic departure within the range of data. The statistical properties of equation 2 in combination with each of the 30 data sets were studied by using the curvature measures of nonlinearity described by Bates and Watts (1), simulation studies, and the bias measure of Box (2). These techniques showed that the nonlinearity was almost totally confined to the parameters b and c, which are of less practical importance than the parameters T_{min} and T_{max}. The estimators of T_{min} and T_{max} were almost unbiased and normally distributed. Evidence that the overall nonlinearity was small was the rapid convergence of the Gauss-Newton algorithm (see Ratkowsky [5] for a full discussion of this).

Equation 2 appears to be a suitable model for the temperature dependence of bacterial growth because it fits the data well and has suitable statistical properties. We have not found a bacterial culture for which this model does not apply. In a previous communication (6), we indicated that T_0 (now T_{min}) values may be useful in categorizing organisms as psychrophiles, psychrotrophs, mesophiles, or thermophiles. The T_{min} values reported for the last three categories were in the expected temperature range. However, the value 276 K computed for *Vibrio psychroerythrus* ATCC 27364 and *Vibrio marinus* ATCC 15381 was not consistent with the description of these organisms as psychrophiles, although the T_{opt} and T_{max} values indicated a psychrophilic nature. Although the proposed relationship (equation 2) allows description of the effect of temperature on the rate of bacterial growth throughout the entire biokinetic range, accurate estimation of T_{min} and T_{max} values can only be obtained if sufficient data are available at temperatures at which the growth rate is low. The cardinal temperatures obtained for *V. marinus* ATCC 15382 suggested that this strain is psychrotrophic and not a "nominal psychrophile" (4).

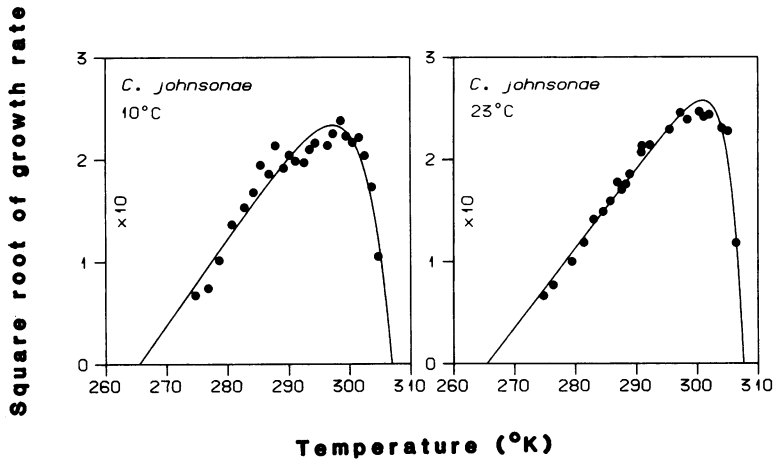

FIG. 3. Data and fitted lines of equation 2 for *C. johnsonae* from Reichardt and Morita (7).

Experiments are in progress on several organisms in an attempt to confirm the biological reality of T_{max}. Preliminary data obtained for *Moraxella* sp. strain 4.16 indicate a maximum temperature for growth within 1°C of the T_{max} value predicted by equation 2. In addition, the reality of T_{min} is being examined for organisms in which this parameter exceeds the freezing point of water.

ACKNOWLEDGMENTS

We thank Phillip Pennington for excellent technical assistance in obtaining the results for *B. stearothermophilus* and *P. morganii* in Table 1. S. Krawiec kindly provided the original data from the paper by Mohr and Krawiec (4). We thank W. Reichardt for providing us with the original data from the paper by Reichardt and Morita (7).

LITERATURE CITED

1. **Bates, D. M., and D. G. Watts.** 1980. Relative curvative measures of nonlinearity. J. R. Stat. Soc. Ser. B **42**:1–25.
2. **Box, M. J.** 1971. Bias in nonlinear estimation. J. R. Stat. Soc. Ser. B **33**:171–201.
3. **Kennedy, W. J., and J. E. Gentle.** 1980. Statistical computing. Marcel Dekker, Inc., New York.
4. **Mohr, P. W., and S. Krawiec.** 1980. Temperature characteristics and Arrhenius plots for nominal psychrophiles, mesophiles and thermophiles. J. Gen. Microbiol. **121**:311–317.
5. **Ratkowsky, D. A.** 1983. Nonlinear regression modeling: a unified practical approach. Marcel Dekker, Inc., New York.
6. **Ratkowsky, D. A., J. Olley, T. A. McMeekin, and A. Ball.** 1982. Relationship between temperature and growth rate of bacterial cultures. J. Bacteriol. **149**:1–5.
7. **Reichardt, W., and R. Y. Morita.** 1982. Temperature characteristics of psychrotrophic and psychrophilic bacteria. J. Gen. Microbiol. **128**:565–568.