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Relationship Between Temperature and Growth Rate of Bacterial Cultures

D. A. RATKOWSKY,[1] JUNE OLLEY,[2] T. A. McMEEKIN,[3]* AND A. BALL[3]

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

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The Arrhenius Law, which was originally proposed to describe the temperature dependence of the specific reaction rate constant in chemical reactions, does not adequately describe the effect of temperature on bacterial growth. Microbiologists have attempted to apply a modified version of this law to bacterial growth by replacing the reaction rate constant by the growth rate constant, but the modified law relationship fits data poorly, as graphs of the logarithm of the growth rate constant against reciprocal absolute temperature result in curves rather than straight lines. Instead, a linear relationship between the square root of growth rate constant (r) and temperature (T), namely, $\sqrt{r} = b(T - T_0)$, where b is the regression coefficient and T_0 is a hypothetical temperature which is an intrinsic property of the organism, is proposed and found to apply to the growth of a wide range of bacteria. The relationship is also applicable to nucleotide breakdown and to the growth of yeast and molds.

Van't Hoff (27) and Arrhenius (2), by analogy to the Van't Hoff thermodynamic equation for chemical equilibrium, put forward the concept that the rate constant for chemical reactions might be suitably described by the following expression in differential form:

$$d \ln k/dT = E/RT^2 \quad (1)$$

where k is the specific reaction rate constant (or simply the rate constant), R is the universal gas constant, T is the absolute temperature, and E is an empirically determined quantity called the activation energy. Upon integration, equation 1 results in the following exponential form:

$$k = A \exp(-E/RT) \quad (2)$$

where the constant A is referred to variously as the "collision factor" or "frequency factor" (16). Equation 2 has become generally known as the Arrhenius Law, and this expression has had some notable success in describing the temperature dependence of chemical reactions.

In microbiology, it has been recognized that temperature is also a cardinal factor controlling the rate of development of microbial populations, and microbiologists have simply substituted growth rate constant r, which is determined assuming an exponential growth model and which is also the reciprocal of the generation time, for rate constant k in equation 2 and have replaced E by a quantity μ which they have called the temperature characteristic. However, although μ is supposed to be a constant in equation 2, there is widespread recognition that it is in fact a decreasing function of temperature (3, 13, 23). The consequence of this is that when $\ln r$ is plotted against reciprocal temperature $1/T$ to produce what is commonly known as an Arrhenius plot, a curve is obtained instead of a straight line. This is readily observed for the six data sets depicted in Fig. 1. This figure, which represents five bacteria and a mold, was redrawn from Johnson et al. (13); it is quite clear that the data do not even remotely approximate a straight-line relationship at any portion of the range. In a more recent paper, Mohr and Krawiec (17) claim that some of their Arrhenius plots show two distinct slopes, but inspection of their Fig. 1 to 3 reveals continuous downward-trending curves for each of their data sets throughout the whole suboptimal temperature range.

The curves of growth rate constant versus temperature as drawn in Fig. 1 are very typical of data for bacterial cultures, as Arrhenius plots of data obtained in the present study (Table 1) and those derived from the literature (Table 2) are all characterized by a continuously changing slope between the minimum and optimum temperatures. A poor fit is generally obtained if one tries to fit the Arrhenius Law to such data, as the response deviates from the linear relationship predicted by equation 2. Laidler (15) has pointed out that the Arrhenius Law is of universal validity for elementary reactions and that "failure to obey the Arrhenius Law, in fact, is an indication

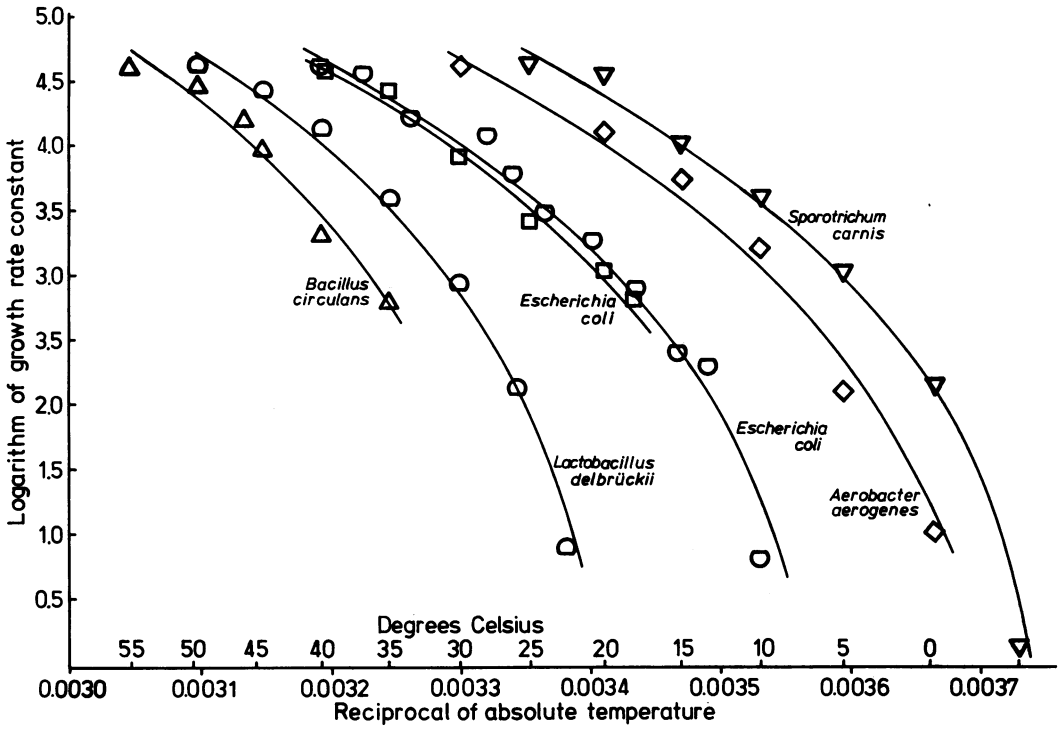

FIG. 1. Arrhenius plot of six sets of data redrawn from Johnson et al. (13). The solid curves correspond to the equation $\sqrt{r} = b\,(T - T_0)$.

that a reaction is not a simple one." Bacterial growth is a complex biological process involving a variety of substrates and enzymes, and it is thus not surprising that the Arrhenius Law does not adequately describe the effect of temperature on the growth of bacteria. In the present work we put forward an alternative linear growth relationship for bacterial cultures growing between the minimum and optimum growth temperatures. In common with the Arrhenius Law as applied to bacterial cultures, there is no theoretical foundation for the alternative relationship to be proposed, but it does at least have the virtue of providing an excellent fit to empirical data.

A relationship of this type was suggested by the work of Ohta and Hirahara (18), who found empirically that a plot of the square root of the rate of nucleotide breakdown in cool-stored carp muscle versus temperature was nearly linear and described by the equation $\sqrt{r} = 0.065\theta + 0.518$, where θ is temperature in

TABLE 1. Sample sizes, correlation coefficients between $\sqrt{r}$ and T, and T_0 values for 14 bacterial cultures

Code no.	Culture	No. of data sets	No. of data points	Avg correlation coefficient	T_0 (mean ± SD)
16L16	*Pseudomonas* group I	15	188	0.991	264.0 ± 2.0
CLD38	*Alteromonas*	6	82	0.996	266.0 ± 1.1
FS1	*Alteromonas*	3	27	0.989	267.8 ± 2.4
FS2	*Alteromonas*	3	21	0.984	263.1 ± 5.5
G489	*Pseudomonas* group IV	9	74	0.991	263.1 ± 1.4
G268	*Pseudomonas* group III	4	31	0.979	272.2 ± 1.9
G249	*Acinetobacter*	4	37	0.968	278.0 ± 1.6
G273	*Acinetobacter*	4	48	0.990	272.5 ± 0.8
G275	*Acinetobacter*	3	20	0.994	277.0 ± 1.4
G281	*Acinetobacter*	2	18	0.976	276.1 ± 2.5
G215	*Micrococcus*	4	41	0.985	273.7 ± 0.7
G274	*Micrococcus*	4	41	0.989	273.6 ± 4.6
G356	Coryneform	4	36	0.988	275.8 ± 3.7
G357	Coryneform	8	54	0.982	278.5 ± 2.9

TABLE 2. Sample sizes, correlation coefficients between $\sqrt{r}$ and T, and T_0 values for cultures in the literature

Culture	Reference	No. of data sets	No. of data points	Correlation coefficient	T_0 (mean ± SD)
Pseudomonas sp. L12	9	1	7	0.998	248
Achromobacter sp.	23	3	20	0.993[a]	261.2 ± 1.5
Pseudomonas sp. L9	9	1	7	0.997	263
Pseudomonas fluorescens	22	6	30	0.996[a]	263.5 ± 2.2
Psychrophilic coliform EBT	3	1	6	0.987	264
Pseudomonas sp. P11	3	1	6	0.988	264
Coliform C1	3	1	6	0.999	265
Coliform C4	3	1	6	0.996	265
Pseudomonas sp. P26	3	1	6	0.992	265
Pseudomonas spp.	11	3	44	0.995[a]	265.1 ± 2.5
Pseudomonas sp.	23	1	6	0.995	266
Pseudomonas sp. P14	3	1	6	0.992	266
Psychrophilic microbacteria	—[b]	3	24	0.995[a]	266.0 ± 0.6
Aerobacter aerogenes	6	1	6	0.993	267
Pseudomonas sp. P22	3	1	6	0.986	269
Pseudomonas sp. P27	3	1	6	0.999	272
Coliform C7	3	1	5	0.989	272
Mesophilic lactobacilli	—[b]	2	11	0.992[a]	272.9 ± 0.2
Coliform C2	3	1	6	0.986	274
Pseudomonas aeruginosa	3	1	6	0.979	274
Coliform C10	3	1	6	0.993	275
Escherichia coli	3	1	6	0.995	275
Pseudomonas aeruginosa	11	1	8	0.995	276
Pseudomonas sp. P15	3	1	6	0.981	276
E. coli	4	1	12	0.992	276
E. coli	14	1	6	0.994	277
E. coli	11	1	15	0.988	280
Lactobacillus delbrueckii	26	1	7	0.994	290
Bacillus circulans	1	1	5	0.989	296

[a] Average correlation coefficients are given when there is more than one data set.
[b] —, Brownlie, thesis.

degrees Celsius. Relationships of this type may be rearranged as follows:

$$\sqrt{r} = b\,(T - T_0) \qquad (3)$$

where b is the slope of the regression line, T is temperature, and T_0 is a conceptual temperature of no metabolic significance. Although T and T_0 may be in degrees Celsius, we choose to use degrees Kelvin to avoid the occurrence of negative temperatures. The growth rates of 14 bacterial cultures were studied over a wide range of temperatures, and the data were used to test the applicability of equation 3.

MATERIALS AND METHODS

The identities of the organisms used are shown in Table 1. Strains of prefixed G were obtained from N. Gillespie, Queensland Fisheries Service, and were isolated from fresh prawns caught at 7 fathoms (ca. 12.8 m) in the G489, which was isolated from spoiled prawns. Other isolates were obtained at the University of Tasmania during the course of other investigations. Strains 16L16 and CLD38 were isolated from spoiled chicken, and FS1 and FS2 were isolated from spoiled fish. The effect of temperature on the growth of these 14 bacterial cultures was examined by using a temperature gradient incubator (Toyo Kagaku Sangyo Co. Ltd., Tokyo, Japan). This permitted examination of growth at approximately 1°C intervals over the range 0 to 44°C. The growth medium (seawater nutrient broth) was inoculated with 0.1 ml of each culture, which had been grown in seawater nutrient broth for 24 h at 22°C. Growth at each temperature was determined by optical density measurements using a nephelometer (EEL Unigalvo). Growth constant r was calculated at each temperature, assessed as the reciprocal of the time taken to reach specific turbidity levels (25, 50, and 79%) or from the slope of curves of the logarithm of turbidity plotted against time. Data sets obtained by all four methods were used to evaluate T_0.

RESULTS AND DISCUSSION

Results were plotted in the form of $\sqrt{r}$ versus T, and excellent straight lines were obtained for temperatures up to or just below the maximum growth rate, beyond which a significant decline occurred in the rate of growth, due to a variety of factors such as inactivation or denaturation of proteins, instability or no synthesis of RNA, or inhibition. Only those data points for which this decline had not yet occurred were

used; this meant that for most data sets the last point or last two points were omitted. Typical data for one organism are plotted in Fig. 2. Results are presented in Table 1. Values of the correlation coefficient between $\sqrt{r}$ and T exceeded 0.97 in 65 of the data sets, and plots of residuals indicated that the data fitted equation 3 well. The other eight data sets had correlation coefficients above 0.93, and none showed any significant deviation from the form of equation 3. Values of T_0 and their standard deviations are also tabulated. Within any single culture the values of T_0 varied little and the means for the cultures examined ranged from 263 to 279°K.

To further examine whether equation 3 was generally applicable to bacterial growth, additional data sets were obtained from the literature (1, 3, 4, 6, 9, 11, 14, 22, 23, 26; L. E. Brownlie, thesis, University of Sydney, Sydney, Australia, 1969). Results are presented in Table 2 in order of increasing T_0 values. All data sets fitted equation 3 excellently with all correlation coefficients exceeding 0.98. Five of these data sets are shown in Fig. 1 in the form of an Arrhenius plot (logarithm of rate versus reciprocal absolute temperature). Curves representing predicted values of the rate obtained from the best-fit lines using equation 3 are superimposed on the data in Fig. 1 and clearly demonstrate that equation 3 closely models the effect of temperature on the growth of each organism between the minimum and optimum values for each organism.

Extrapolation of the regression line obtained by plotting $\sqrt{r}$ versus T yields the temperature T_0 at the point where the line intersects the temperature axis. It should be noted that the minimum growth temperature is only a hypothetical concept since equation 3 is valid only at temperatures where water activity is not changing due to ice formation (24). From Table 2 the psychrophile described by Harder and Veldkamp (9) has a T_0 value of 248°K, and the psychrotrophs have values in the region between 261 and 269°K. Of interest are the results of six data sets on *Pseudomonas fluorescens* (22), these being obtained from factorial combinations of three growth media and two conditions of aeration. The T_0 values were independent of medium and aeration, indicating that T_0 is an intrinsic property of the organism when growth conditions other than temperature are nonlimiting. T_0 values for mesophiles were intermediate, i.e., from approximately 270 to 280°K, between psychrotrophs and thermophiles. The two thermophiles in Table 2 have T_0 values of 290 and 296°K respectively. T_0 values may therefore be a useful aid in addition to optimum growth temperature to categorize a microorganism as a psychrophile, psychrotroph, mesophile, or thermophile. The data presented in Tables 1 and 2 indicate distinct T_0 values for psychrophiles and thermophiles with a gradation from psychrotrophs to mesophiles. It seems likely that as further T_0 values are determined there will be a continual gradation of organisms across the spectrum from psychrophile to thermophile. Previously, attempts to characterize the temperature relationships of microorganisms have been derived from Arrhenius plots. Thus Ingraham (11, 12) proposed that the temperature characteristic (μ) could be used to determine whether an organism was a psychrophile or mesophile. This concept was challenged by Hanus and Morita (8), who found no significant correlation between μ values of psychrophiles, psychrotrophs, and mesophiles. A similar conclusion was drawn by Shaw (25) for yeasts and by Herbert and Bhakoo (10) for five psychrophilic vibrios. Since the Arrhenius Law does not adequately describe the temperature dependence of bacterial cultures, as emphasized in the introduction to this paper, it is not surprising to find that μ may vary as much as threefold or fourfold throughout a single set of data depending upon which portion of the data set is used. This problem does not arise when equation 3 is used, as it applies throughout the whole range of the response from the minimum to the optimum values.

It therefore appears that equation 3 may be used to describe the relationship between temperature and growth rate of microorganisms between the minimum and optimum temperatures and may be used instead of the Arrhenius Law. The relationship may find application in other areas of biological science. As an example, other investigations in our laboratories have shown the relationship to describe the effect of

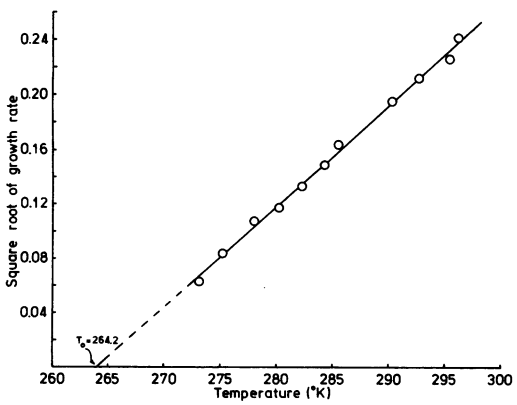

FIG. 2. Typical linear relationship for *Pseudomonas* group 1 strain 16L16 between square root of growth rate and temperature. Growth rate was measured as the reciprocal of the time to reach 25% turbidity.

temperature on the deterioration of proteinaceous foods. This might be expected, since nucleotide breakdown (18) which precedes spoilage has been shown to obey equation 3 with a T_0 of 265°K. This T_0 value is similar to that obtained for many pseudomonads which are the major spoilage organisms of proteinaceous foods stored aerobically at chill temperatures. Under these conditions psychrotrophic pseudomonads are selected because they have generation times up to 30% faster than competitors (5). Temperature is the cardinal factor controlling the rate of growth since other factors such as nutrient status and available water are nonlimiting and no microbial interactions occur until maximum cell densities are reached (5). Therefore a knowledge of the effect of temperature on the rate of growth of the spoilage flora may be used to monitor the time-temperature history of expired shelf life of the product. This process, temperature function integration, is accomplished by use of electronic integrators of which the circuitry contains the relationship between growth rate and temperature (19). To date this information has been based upon the empirical relative rate curve constructed by Olley and Ratkowsky (20, 21) from 70 data sets in the literature. The empirical curve can now be replaced by a relative rate curve calculated from equation 3. A T_0 value of 263°K, which is close to the lowest value obtained for typical psychrotrophic pseudomonads, gives a relative rate curve which is in excellent agreement with the empirical data.

A further use of equation 3 is that it accurately describes the data (23) on the growth of yeast species of the genera *Candida*, *Geotrichoides*, and *Mycotorula*, with T_0 values near 260, and it also accurately describes the growth of the mold *Sporotrichum carnis* (7) with $T_0 = 264$ (this latter set of data is shown in Fig. 1).

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