

Temperature Characteristics and Arrhenius Plots for Nominal Psychrophiles, Mesophiles and Thermophiles

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The specific growth rates at various temperatures of 12 bacterial species were measured and plotted as Arrhenius profiles. Temperature characteristics and optimum temperatures for maximum specific growth rates were estimated from these curves. The data reveal that one of two forms of the Arrhenius profile is characteristic of each bacterium: one curve is a simple smooth curve with a single predominant slope at sub-optimum temperatures; the other is a more complex curve with two distinct slopes at sub-optimum temperatures. The simple curve describes bacteria across the entire biokinetic range whereas the more complex curve occurs only with organisms which have optimum temperatures for replication above 37 °C. Describing bacteria in terms of these forms of the Arrhenius profile is less arbitrary than is categorization based on optimum temperatures.

INTRODUCTION

A comparative examination of the rates of replication of 12 species of bacteria has revealed two distinct temperature-dependent patterns. The notable features of the investigation reported here are that: (1) the organisms selected include representatives from all the classes that have been defined on the basis of minimum, optimum and maximum temperatures for replication, namely, psychrophiles, psychrotrophs, mesophiles, stenothermophiles, eurythermophiles and extreme thermophiles (Lamanna *et al.*, 1973); (2) all the organisms were grown in similar media, thereby minimizing a variable which is known to affect the temperature characteristic (the logarithmic proportionality between specific growth rate and the reciprocal of environmental temperature); (3) the data are presented as Arrhenius curves; and (4) analyses have been applied to the data to establish empirical relationships between optimum temperature(s) for replication and temperature characteristic(s).

The forms of Arrhenius curves for the psychrophiles, psychrotrophs, some mesophiles and some thermophiles are similar. Thus, categorizing these organisms into distinct classes by their positions in a temperature spectrum is arbitrary. Furthermore, the forms of the Arrhenius curves for some mesophiles and some thermophiles are more complex than those for the psychrophiles and other mesophiles and thermophiles. The two recurrent forms of the Arrhenius curve establish uncontrived categories which can be used to classify the temperature-dependent rates of replication of bacteria.

METHODS

Source of organisms. *Vibrio psychroerythrus* ATCC 27364, *V. marinus* ATCC 15381 and *V. marinus* ATCC 15382 were obtained from the American Type Culture Collection; *V. marinus* has been listed as *V. fischeri* in the twelfth edition of the ATCC catalogue, but the strain numbers have been retained. *Thermus*

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aquaticus strain TY was provided by Professor T. D. Brock, University of Wisconsin (Madison), U.S.A. *Pseudomonas fluorescens*, *P. aeruginosa*, *Serratia marcescens*, *Escherichia coli*, *Bacillus subtilis*, *B. megaterium*, *B. coagulans* and *B. stearothermophilus* were taken from stocks maintained at Lehigh University.

Media. All organisms except *V. psychroerythrus* were maintained and grown on the basal salts medium described by Brock & Freeze (1969). For the *V. marinus* strains, NaCl was added to a final concentration of 3.5% (w/v). *Vibrio psychroerythrus* was grown in medium containing 0.5 M-NaCl, 0.3 M-MgCl₂, 0.4 mM-KH₂PO₄, 0.01 μM-FeCl₂, 0.15 mM-CaCl₂ and 0.8% (w/v) tryptone; pH 7.0 (D'Aoust & Kushner, 1971).

Growth. Growth was monitored spectrophotometrically at 660 nm (Bausch and Lomb, Spectronic 20). Erlenmeyer flasks (300 ml) with cuvettes fused to the sides and containing 25 ml of the appropriate medium were inoculated from an overnight culture or, in the case of psychrophiles, a 48 to 72 h culture. The initial turbidity was generally between 0.02 and 0.05. The cultures were incubated in a rotary water-bath shaker (New Brunswick, model G76) operating at 180 rev. min⁻¹. Temperatures were maintained within ±0.5 °C. For temperatures below ambient, a low temperature circulator was connected to the water-bath shaker. Turbidity was measured at 15, 30 or 60 min intervals and plotted against time. Regression analyses were performed for the exponential phase of growth.

Arrhenius plots were constructed by plotting log_e(10⁸ × *k*) against 10³ × 1/*T*, where *k* is the specific growth rate (h⁻¹) and *T* is the absolute temperature (K). The specific growth rates at each temperature were calculated from at least two growth curves or, more typically, from three to six growth curves. Temperature characteristics were determined from the slopes of the linear portion of the Arrhenius curves at sub-optimum temperatures. In those instances in which there were two distinct slopes at sub-optimum temperatures, the data points were grouped to produce the highest sum of *r*² values for the two lines.

RESULTS AND DISCUSSION

A modified version of the Arrhenius equation

$$k = Ae^{-\mu/RT} \quad (1)$$

in which μ , a temperature characteristic, is substituted for E_a , an activation energy, describes the specific growth rate, *k*, of bacteria over a limited temperature range (cf. Ingraham, 1962). Unlike enzymic reactions, bacterial cells are holistic systems which need not be viewed as a catenary sequence of reactions controlled by one or more master reactions. Furthermore, they are not dilute, homogeneous, nor wholly aqueous. Nonetheless, equation 1 is an accurate statement of the temperature-dependence of the specific growth rate over a large range of sub-optimum temperatures.

The specific rate of replication at any temperature (between the maximum for growth, T_{max} , and the minimum, T_{min}) can be described as the overall temperature-dependent rate(s) of synthesis *minus* the overall temperature-dependent rate of degradation (Allen, 1953; Dean & Hinshelwood, 1966). For example,

$$k = A_1e^{-\mu_1/RT} - A_2e^{-\mu_2/RT} \quad (2)$$

is a formulation which provides an accurate statement of specific growth rates at most temperatures for many bacteria. Unfortunately, a manner in which the values of A_1 , A_2 , μ_1 and μ_2 (in equation 2) can be readily and accurately obtained has not been established (Dean & Hinshelwood, 1966). But, μ_1 can be estimated from the negative slopes in the Arrhenius profiles and μ_2 from the positive ones.

The Arrhenius plots for *V. psychroerythrus* and the two *V. marinus* strains (nominally psychrophiles) are presented in Fig. 1; those for *S. marcescens*, *P. fluorescens*, *E. coli*, *B. subtilis*, *B. megaterium* and *P. aeruginosa* (nominally mesophiles) in Fig. 2; and those for *B. stearothermophilus*, *B. coagulans* and *T. aquaticus* (thermophiles) in Fig. 3. Optimum temperatures were determined by inspection for maximum growth rates on Arrhenius plots. Values for μ were calculated by regression analyses for the one or two linear portions of the curve that occur below the optimum temperature. The optimum temperatures and the temperature characteristics for the 12 organisms are summarized in Table 1.

The Arrhenius profiles for *V. psychroerythrus*, *V. marinus*, *S. marcescens*, *P. fluorescens* and *T. aquaticus* (Figs 1, 2 and 3) can be described by equation 2. Thus, this pattern of temperature-dependent growth occurs over the entire biokinetic range.

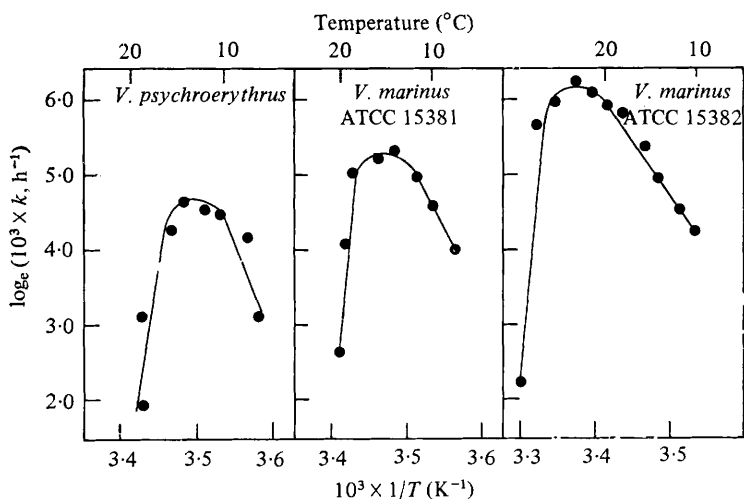


Fig. 1. Arrhenius curves for *V. psychroerythrus*, *V. marinus* 15381 and *V. marinus* 15382. The r^2 values for the slopes from which μ_1 values were calculated were 0.93 for *V. psychroerythrus*, 0.99 for *V. marinus* 15381, and 0.98 for *V. marinus* 15382.

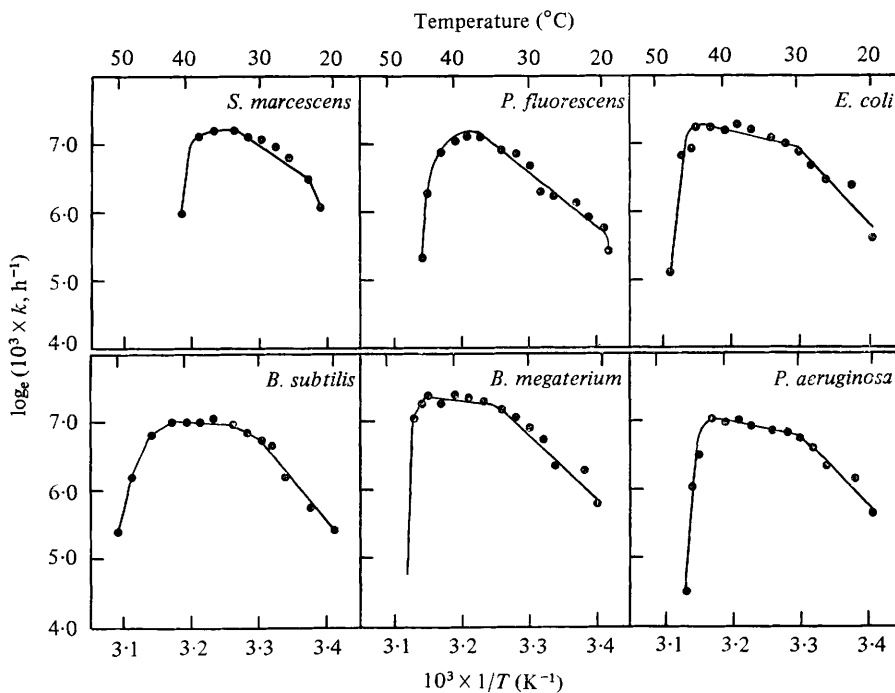


Fig. 2. Arrhenius curves for *S. marcescens*, *P. fluorescens*, *E. coli*, *B. subtilis*, *B. megaterium* and *P. aeruginosa*. The r^2 values for the slopes from which μ_1 values were calculated were 0.96 for *S. marcescens* and 0.98 for *P. fluorescens*. The r^2 values for the slopes from which the μ_1 and μ_1' values were calculated were, respectively, 0.32 and 0.99 for *B. subtilis*, 0.56 and 0.97 for *B. megaterium*, 0.95 and 0.96 for *P. aeruginosa*, and 0.85 and 0.87 for *E. coli*.

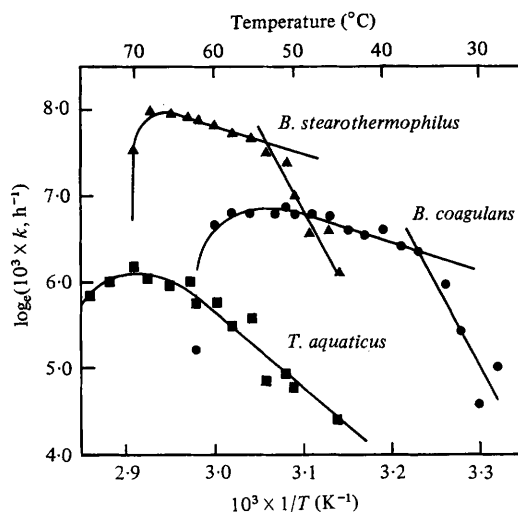


Fig. 3. Arrhenius curves for *T. aquaticus* (■), *B. coagulans* (●) and *B. stearothermophilus* (▲). The r^2 value for the slope from which μ_1 of *T. aquaticus* was calculated was 0.95. The r^2 values for the slopes from which the μ_1 and μ_1' values were calculated were, respectively, 0.97 and 0.92 for *stearothermophilus* and 0.93 and 0.88 for *B. coagulans*.

Table 1. Optimum temperature for growth and temperature characteristics of selected prokaryotes

Organism	Optimum temperature (°C)	Temperature characteristics*	
		μ_1 (cal)†	μ_1' (cal)
<i>Vibrio psychroerythrus</i>	13.8	51 900	
<i>V. marinus</i> ATCC 15381	15.5	39 000	
<i>V. marinus</i> ATCC 15382	24.0	23 200	
<i>Serratia marcescens</i>	34.5	12 700	
<i>Pseudomonas fluorescens</i>	37.9	15 500	
<i>Bacillus subtilis</i>	38.1	900	24 700
<i>B. megaterium</i>	39.6	2 400	17 800
<i>Pseudomonas aeruginosa</i>	42.3	3 700	18 400
<i>Escherichia coli</i>	44.5	4 400	20 300
<i>Bacillus coagulans</i>	53.9	6 500	30 300
<i>B. stearothermophilus</i>	65.8	6 600	30 100
<i>Thermus aquaticus</i>	70.2	17 800	

* The r^2 values for the slopes from which μ_1 values were calculated were significant at the level of 0.995 for *V. marinus* 15382, *S. marcescens*, *P. fluorescens*, *P. aeruginosa*, *E. coli*, *B. coagulans*, *B. stearothermophilus* and *T. aquaticus*, and at the level of 0.95 for *V. marinus* 15381; the values for *V. psychroerythrus*, *B. subtilis* and *B. megaterium* are significant at lower levels. The r^2 values for the slopes from which μ_1' values were calculated were significant at the level of 0.995 for *B. subtilis*, *B. megaterium*, *P. aeruginosa*, *B. coagulans* and *B. stearothermophilus*, and at the level of 0.95 for *E. coli*.

† 1 cal = 4.184 J.

The Arrhenius profiles for some mesophiles and thermophiles have two slopes at sub-optimum temperatures (Figs 2 and 3). The point which is common to both slopes has been called the 'critical temperature', T_{crit} (Lamanna *et al.*, 1973). Equations 1 and 2 can be combined to describe the more complex pattern of growth. One combination is

$$k = A_1 e^{-\mu_1/RT} - A_2 e^{-\mu_2/RT} \quad T_{crit} < T < T_{max} \quad (3)$$

$$= A_1' e^{-\mu_1'/RT} \quad T_{min} < T < T_{crit} \quad (4)$$

in which μ_1' is the temperature characteristic for growth between T_{crit} and T_{min} , and μ_1 is the temperature characteristic for growth between T_{crit} and the optimum temperature. These relationships imply that the organism undergoes a discrete change in organization at T_{crit} , that is, equation 3 describes temperature-dependent growth at temperatures greater than T_{crit} and equation 4 describes temperature-dependent growth below T_{crit} . A contrasting description is that a balance of organizations exists at any temperature. This condition can be shown as

$$k = \frac{A_1 e^{-\mu_1/RT} \cdot A_1' e^{-\mu_1'/RT}}{A_1 e^{-\mu_1/RT} + A_1' e^{-\mu_1'/RT}} - A_2 e^{-\mu_2/RT} \quad (5)$$

Equation 5 can be reduced to

$$k = \frac{1}{A_1^* e^{\mu_1'/RT} + A_1^{**} e^{\mu_1/RT}} - A_2 e^{-\mu_2/RT} \quad (6)$$

in which $A_1^* = 1/A_1'$ and $A_1^{**} = 1/A_1$.

Inspection of the Arrhenius profiles for *E. coli*, *B. subtilis*, *B. megaterium* and *P. aeruginosa* reveals that these organisms have Arrhenius profiles with two discrete slopes at sub-optimum temperatures (Fig. 2). This form of the profile is more pronounced with some thermophiles – in particular, with *B. coagulans* and *B. stearothermophilus* (Fig. 3). Our data show that the more complex curves describe the growth of both Gram-negative and Gram-positive organisms. Complex curves occur exclusively but not necessarily with organisms having optimum temperatures above 37 °C.

The disparate Arrhenius profiles and the differences between equation 2 and equations 3 and 4, or between equation 2 and equation 6, indicate that different patterns of temperature-dependent growth occur. These formulations might exclude some proposed mechanisms of temperature-dependent growth but they cannot specify the actual mechanisms. That several organizational states exist among the thermophiles is shown by the various positions of Arrhenius curves in the field established by the temperature and growth rate coordinates. Organisms with similar optimum temperatures, e.g. *B. stearothermophilus* and *T. aquaticus*, differ in their temperature characteristics and markedly so in their maximum rates of replication.

Generally speaking, there appears to be good agreement among corrected values of μ_1 calculated in different laboratories despite differences in strains, media and some physical growth conditions. Hanus & Morita (1968) report μ values for *V. marinus* isolates using the base 10 while our values employ the base e. Close agreement becomes apparent when their values are multiplied by 2.303 to correct for this difference. Similarly, Ingraham (1958) reported a temperature characteristic for *E. coli*, the calculation of which omitted the gas factor (cf. Hanus & Morita, 1968). Multiplying his value by 1.987 to correct for the omission leads to good agreement with the value we report. Ward & Cockson (1972) calculated a temperature characteristic of 26400 cal for a Gram-positive, endospore-forming rod which appears to be a strain of *B. stearothermophilus*. Their value is based on growth rates at four sub-optimum temperatures and is one that falls between the two temperature characteristics we obtained by measuring growth rates at 12 sub-optimum temperatures. The 17000 cal temperature characteristic observed for *Myxococcus xanthus*, which has an optimum temperature for replication of 34 to 36 °C, is similar in magnitude to the value we observed for bacteria with optimum temperatures in this range (Janssen *et al.*, 1977). Also, the temperature characteristics of 15000 and 13500 cal in minimal and rich media, respectively, for an unidentified bacillus (Epstein & Grossowicz, 1969) with an optimum temperature from 55 to 58 °C is consistent with our observations (see below).

In this study, estimates of the temperature characteristics for organisms with optimum temperatures across a major portion of the biokinetic range were obtained. These data allow formulation of empirical relationships between the optimum temperatures for replication

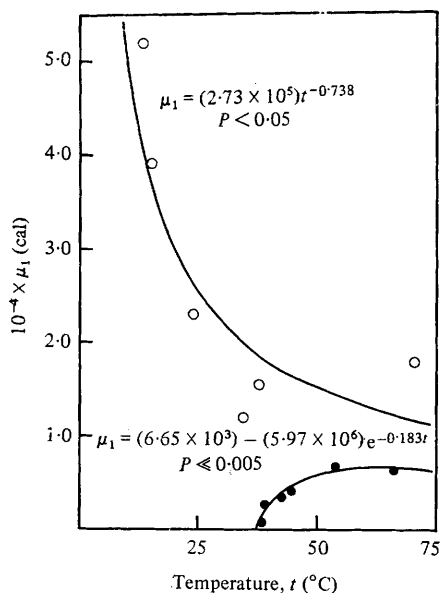


Fig. 4. Empirical relations between μ_1 values and the optimum temperatures for growth: \circ , μ_1 values for organisms which have a single predominant slope in their Arrhenius profiles at sub-optimum temperatures; \bullet , μ_1 values for organisms with two distinct slopes in their Arrhenius profiles at sub-optimum temperatures.

and temperature characteristics. Figure 4 presents all μ_1 values which we have estimated. It may be statistically improper to include in a single regression μ_1 values from organisms with different forms of the Arrhenius profile, i.e. from organisms with two distinct slopes at sub-optimum temperatures as opposed to those with a single slope. Accordingly, two curves have been drawn; the upper line (\circ) relates the optimum temperature (t , $^{\circ}\text{C}$) and μ_1 values (cal) for those organisms described by equation 2; the lower line (\bullet) relates the optimum temperature and μ_1 values for those organisms described by equations 3 and 4, or equation 6. The upper curve of Fig. 4 is best described (Crowe & Crowe, 1969) by the empirical equation:

$$\mu_1 = (2.73 \times 10^5)t^{-0.738} \quad (7)$$

The lower curve best fits the empirical equation (Crowe & Crowe, 1969):

$$\mu_1 = (6.65 \times 10^3) - (5.97 \times 10^6)e^{-0.183t} \quad (8)$$

At 37.2°C , the μ_1 value becomes zero indicating that only organisms with an optimum temperature of approximately this value or greater can have two distinct temperature characteristics at sub-optimum temperatures.

The observations presented in this report indicate that the temperature-dependent specific growth rates of selected bacteria occur in two patterns. Each of these patterns can be expressed as a variation of the Arrhenius equation. Furthermore, for each pattern of growth, the temperature characteristics (which describe the rate of change of specific growth rate in a range of temperatures just below the optimum) vary in a regular but non-linear manner relative to optimum temperature.

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REFERENCES

- ALLEN, M. B. (1953). The thermophilic aerobic sporeforming bacteria. *Bacteriological Reviews* **17**, 125-173.
- BROCK, T. D. & FREEZE, H. (1969). *Thermus aquaticus* gen.n. and sp.n., a non-sporulating extreme thermophile. *Journal of Bacteriology* **98**, 289-297.
- CROWE, A. & CROWE, A. (1969). *Mathematics for Biologists*. New York: Academic Press.
- D'Aoust, J. Y. & KUSHNER, D. J. (1971). Structural changes during lysis of a psychrophilic marine bacterium. *Journal of Bacteriology* **108**, 916-927.
- DEAN, A. C. R. & HINSELWOOD, C. (1966). *Growth, Function and Regulation in Bacterial Cells*. London: Oxford University Press.
- EPSTEIN, I. & GROSSOWICZ, N. (1969). Prototrophic thermophilic *Bacillus*: isolation, properties, and kinetics of growth. *Journal of Bacteriology* **99**, 414-417.
- HANUS, F. J. & MORITA, R. Y. (1968). Significance of the temperature characteristic of growth. *Journal of Bacteriology* **95**, 736-737.
- INGRAHAM, J. L. (1958). Growth of psychrophilic bacteria. *Journal of Bacteriology* **76**, 75-80.
- INGRAHAM, J. L. (1962). Temperature relationships. In *The Bacteria*, vol. 4, pp. 265-296. Edited by I. C. Gunsalus & R. Y. Stanier. New York: Academic Press.
- JANSSEN, G. R., WIREMAN, J. W. & DWORKIN, M. (1977). Effect of temperature on the growth of *Myxococcus xanthus*. *Journal of Bacteriology* **130**, 561-562.
- LAMANNA, C., MALLETTE, M. F. & ZIMMERMAN, L. N. (1973). *Basic Bacteriology*, 4th edn. Baltimore: Williams & Wilkins.
- WARD, J. & COCKSON, A. (1972). Studies on a thermophilic bacillus: its isolation, properties, and temperature coefficient of growth. *Journal of Bacteriology* **112**, 1040-1042.