Relationship Between Temperature and Growth Rate of Bacterial Cultures

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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 regres-
sion 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:

\[
d \ln k/dT = E/RT^2
\]  

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:

\[
k = A \exp (-E/RT)
\]  

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 tempera-
ture dependence of chemical reactions.

In microbiology, it has been recognized that
temperature is also a cardinal factor controlling
the rate of development of microbial popula-
tions, and microbiologists have simply substitut-
ed 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 \( \mu \) which they have
called the temperature characteristic. However,
although \( \mu \) 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 re-
drawn 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 downwar-
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 tem-
peratures. 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 validi-
ity for elementary reactions and that “failure to
obey the Arrhenius Law, in fact, is an indication
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 cooled stored carp muscle versus temperature was nearly linear and described by the equation \( \sqrt{\tau} = 0.0650 + 0.518 \), where \( \tau \) is temperature in

![Arrhenius plot of six sets of data redrawn from Johnson et al. (13). The solid curves correspond to the equation \( \sqrt{\tau} = b (T - T_0) \).](image)

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

### TABLE 1. Sample sizes, correlation coefficients between \( \sqrt{\tau} \) and \( T \), and \( T_0 \) values for 14 bacterial cultures

<table>
<thead>
<tr>
<th>Code no.</th>
<th>Culture</th>
<th>No. of data sets</th>
<th>No. of data points</th>
<th>Avg correlation coefficient</th>
<th>( T_0 ) (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16L16</td>
<td><em>Pseudomonas</em> group I</td>
<td>15</td>
<td>188</td>
<td>0.991</td>
<td>264.0 ± 2.0</td>
</tr>
<tr>
<td>CLD38</td>
<td>Alteromonas</td>
<td>6</td>
<td>82</td>
<td>0.996</td>
<td>266.0 ± 1.1</td>
</tr>
<tr>
<td>FS1</td>
<td>Alteromonas</td>
<td>3</td>
<td>27</td>
<td>0.989</td>
<td>267.8 ± 2.4</td>
</tr>
<tr>
<td>FS2</td>
<td>Alteromonas</td>
<td>3</td>
<td>21</td>
<td>0.984</td>
<td>263.1 ± 5.5</td>
</tr>
<tr>
<td>G489</td>
<td><em>Pseudomonas</em> group IV</td>
<td>9</td>
<td>74</td>
<td>0.991</td>
<td>263.1 ± 1.4</td>
</tr>
<tr>
<td>G268</td>
<td><em>Pseudomonas</em> group III</td>
<td>4</td>
<td>31</td>
<td>0.979</td>
<td>272.2 ± 1.9</td>
</tr>
<tr>
<td>G249</td>
<td>Acinetobacter</td>
<td>4</td>
<td>37</td>
<td>0.968</td>
<td>278.0 ± 1.6</td>
</tr>
<tr>
<td>G273</td>
<td>Acinetobacter</td>
<td>4</td>
<td>48</td>
<td>0.990</td>
<td>272.5 ± 0.8</td>
</tr>
<tr>
<td>G275</td>
<td>Acinetobacter</td>
<td>3</td>
<td>20</td>
<td>0.994</td>
<td>277.0 ± 1.4</td>
</tr>
<tr>
<td>G281</td>
<td>Acinetobacter</td>
<td>2</td>
<td>18</td>
<td>0.976</td>
<td>276.1 ± 2.5</td>
</tr>
<tr>
<td>G215</td>
<td><em>Micrococcus</em></td>
<td>4</td>
<td>41</td>
<td>0.985</td>
<td>273.7 ± 0.7</td>
</tr>
<tr>
<td>G274</td>
<td><em>Micrococcus</em></td>
<td>4</td>
<td>41</td>
<td>0.989</td>
<td>273.6 ± 4.6</td>
</tr>
<tr>
<td>G356</td>
<td>Coryneform</td>
<td>4</td>
<td>36</td>
<td>0.988</td>
<td>275.8 ± 3.7</td>
</tr>
<tr>
<td>G357</td>
<td>Coryneform</td>
<td>8</td>
<td>54</td>
<td>0.982</td>
<td>278.5 ± 2.9</td>
</tr>
</tbody>
</table>
TABLE 2. Sample sizes, correlation coefficients between $\sqrt{r}$ and $T$, and $T_0$ values for cultures in the literature

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

$^a$ 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:

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

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 the curve 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 ($\mu$) 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 $\mu$ 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 $\mu$ 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.](http://jb.asm.org/)
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$^\circ$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$^\circ$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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LITERATURE CITED