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Dimensionless analysis of the microbial growth rate dependence on sub-optimal temperatures

P Dantigny

Laboratoire de Microbiologie, UA INRA, ENSBANA, 1 Esplanade Erasme, F-21000 Dijon, France

The influence of sub-optimal temperatures (T) on the microbial growth rate (μ) has been assessed by means of dimensionless variables: $T_{dim} = [T–T_{min}]/[T_{opt}–T_{min}]$ and $\mu_{dim} = \mu/\mu_{opt}$. T_{min} represents the temperature at which there is no growth, T_{opt} the optimum temperature at which the growth rate, μ_{opt}, is maximum. Data sets, growth rate *vs* temperature, have been taken from the literature for 12 organisms (psychrotrophs, mesophiles and thermophiles). In order to compare these organisms, the power law function has been used: $[\mu_{dim}] = [T_{dim}]^{\alpha}$. The parameters μ_{opt} and T_{opt} are determined from direct readings whereas T_{min} and α are estimated by means of a non-linear regression. An accurate estimation of T_{min} is obtained providing low growth rate data are available. A wide range of optimal temperatures where the growth rate almost equals μ_{opt} prevents one from obtaining a narrow confidence interval for α. On the basis of the analysis hereafter developed, thermophiles are characterized by values of the power α less than mesophiles and psychrotrophs. Almost all of these values are significantly different from two, previously determined for *Staphylococcus xylosus* and widely used for predicting the microbial growth in foods.

Keywords: model; temperature; growth rate; dimensionless

Introduction

Since the early work of Barber [4], the influence of temperature on microbial growth rate has received much attention. Amongst the environmental factors, temperature is an important parameter to monitor for process optimization and for food preservation. Temperature is also of fundamental interest in taxonomy, for organisms are classified into distinct classes by their positions in a temperature spectrum. Although temperature is a parameter likely to vary during processes or storage, most of the publications describe results at constant temperatures. From the minimum temperature for growth, T_{min}, the growth rate increases with increasing temperature until close to optimal. Then the curve, growth rate *vs* temperature, flattens and the growth rate is almost constant. At temperatures higher than T_{opt}, the growth rate decreases sharply. The Arrhenius law was originally proposed to describe the temperature dependence of the specific growth rate and has been applied by many workers [12,14,15,18,19,29]. In order to account for the non-linearity of the relationship between the temperature and the growth rate, the Arrhenius law was altered [27,28]. In 1980, Mohr and Krawiec [18] noticed that the forms of Arrhenius curves for psychrophiles, psychrotrophs, some mesophiles and some thermophiles are similar. These authors have attempted to classify organisms by claiming two distinct slopes for some mesophiles and thermophiles. Later, in 1982, these observations were rejected [23] and the square-root model was suggested by Ratkowsky *et al* [24]. This empirical model is based upon the observation that the square-root of nucleotide degradation in carp muscle is related to temperature [20]. The square-root model is widely used in predictive microbiology.

There is a need for a tool allowing all data to be compared. On one hand, there are different methods for calculating the growth rate (eg, reciprocal of generation time, reciprocal of time to reach a certain density, reciprocal of time to obtain a certain multiplication factor, or μ_m of the Gompertz equation). In addition, there is sometimes confusion between the growth rate and the specific growth rate [10]. On the other hand, the temperature unit can be Celsius, Kelvin, or even Farenheit. Moreover, the organisms are grown on different media, in different environments. It is traditional procedure in bioprocessing to use dimensionless groups (variables with no units) to establish correlation (ie, heat and mass transfer) and to exhibit different behaviors of processes (ie, fluid dynamics). In addition, it is of particular interest for fitting purposes to have these variables normalized, varying within the range 0 to 1. A dimensionless approach has been used to describe the decrease of the cellular yield and the increase of the yield ethanol over glucose by increasing the dilution rate in a chemostat fermentation of *Saccharomyces cerevisiae* on glucose [7]. The normalized yields and dilution rate, were then included into the equation set of a structured mechanistic model capable of describing the growth on mixed substrates [8] and during the transition from ethanol oxidation to glucose utilization [9]. Characteristic numbers have also been used for describing the influence of the environment on microorganisms [26,30].

This paper compares 12 organisms (four thermophiles, three mesophiles, four psychrotrophs, and *Acinetobacter* which is considered either mesophilic [3] or psychrotrophic [16]), using the following dimensionless variables: $T_{dim} = [T–T_{min}]/[T_{opt}–T_{min}]$ and $\mu_{dim} = \mu/\mu_{opt}$. For an easy discrimination the power-law function has been selected: $[\mu_{dim}] = [T_{dim}]^{\alpha}$. This model was originally described by

Correspondence: Dr P Dantigny, Laboratoire de Microbiologie, UA INRA, ENSBANA, 1 Esplanade Erasme, F-21000 Dijon, France
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Bělehrádek [5,6], who found that the power differs from one biological reaction to another. Subsequently, it was found that the square-root model is a particular case of the Bělehrádek model [25]. McMeekin *et al* [17] demonstrated that the α value of two can be used for *Staphylococcus xylosus*. By extension this value has been applied to other organisms such as *Listeria monocytogenes* [10], *Yersinia enterolitica* [1,2] and *Clostridium botulinum* [11]. In contrast to the square-root model, the power is not necessarily equal to two, but a design parameter to be estimated. Categorization of the organisms will be based on the value of α.

Materials and methods

The identities of the organisms selected are listed in Table 1. The organisms have been sorted by ascending optimum temperature. The minimum number of data required for selection has been set to 10. Graphs have been scanned, then individual points have been digitized by means of software (UnGraph 4.0, BioSoft, Cambridge, UK). The data obtained are available on request from the author. The dimensionless approach is based upon the observation of an increase in the growth rate, by increasing the temperature from the minimum temperature, T_{min}, at which the growth rate is zero, to the optimum temperature, T_{opt}. The growth rate at T_{opt} is noted μ_{opt}. The variables of the model, μ_{dim} and T_{dim}, as defined below,

$$\mu_{dim} = \frac{\mu}{\mu_{opt}} \quad (1)$$

$$T_{dim} = \frac{T - T_{min}}{T_{opt} - T_{min}} \quad (2)$$

represent the dimensionless growth rate and the dimensionless temperature, respectively. The power-law function for modeling the growth rate at sub-optimum temperature is:

$$[\mu_{dim}] = [T_{dim}]^{\alpha} \quad (3)$$

By substituting the dimensionless variables for their definition, we found:

$$\left[\frac{\mu}{\mu_{opt}}\right] = \left[\frac{T - T_{min}}{T_{opt} - T_{min}}\right]^{\alpha} \quad (4)$$

In order to stabilize the variance of the growth rate, a logarithmic transformation has been used as suggested by Alber and Schaffner [1].

$$\ln(\mu) = \ln(\mu_{opt}) + \alpha[\ln(T - T_{min}) - \ln(T_{opt} - T_{min})] \quad (5)$$

In most cases, the optimum temperature for the organisms is stated in the paper, for example Mohr and Krawiec [18]. Otherwise, the optimum temperature is defined as the temperature at which the growth rate is maximum, this method requires data over the full kinetic range. The optimum temperature for *Clostridium botulinum* type B given by the authors [11] is consistent with a range of T_{opt} values reported in the literature [13]. The optimal growth rate is evaluated at the optimal temperature. When more than one datum is obtainable at the optimum temperature, the average of all growth rates is taken. When no growth rate is available at T_{opt}, the optimum growth rate is taken at the closest tempeature. T_{min} is a biological parameter, but its value differs greatly from one strain to another; it depends upon the medium composition, upon the organism's physiology, and is not easily attainable. Therefore, T_{min} has been estimated along with α by a non-linear regression software based upon the Levenberg–Marquardt Algorithm (SlideWrite 3.0, Advanced Graphics Software, Carlsbad, CA, USA). The coefficients of the non-linear fitting function are determined by an iterative process minimizing the chi-squared merit function (least squares criterion). The Gauss–Jordan method is employed for matrix inversion at each iteration. The initial parameter values were set to two for α, and for a convergence purpose at 2°C below the lowest temperature data for T_{min}.

Results and discussion

The results of the estimation procedure are reported in Table 2. Firstly, for most of the organisms evaluated, the 95% confidence intervals for the minimum temperature is about ±2°C. These results are similar to most of the models where T_{min} is a parameter to be estimated. The range of *B. coagulans*, ±3.5°C, is a little bit greater. The range of *T. aquaticus*, ±24.4°C exhibits clearly the lack of fit of the model in that case. It has been reported that the minimum temperature for the growth of *T. aquaticus* and *Aeromonas*

Table 1 List and references of the selected organisms. Determination of the optimum parameters

Code	Organism	Number of data	Reference	μ_{opt} (h^{-1})	T_{opt} (°C)
Ps	*Pseudomonas* 16L16	19	[24]	1.26	28.5
Ac	*Acinetobacter* 4.41	22	[24]	0.87	29.0
Ae	*Aeromonas* 4.29	17	[24]	0.87	34.0
CB	*Clostridium botulinum* type B	10	[11]	1.87	35.0
LP	*Lactobacillus plantarum*	80	[31]	1.27	37.0
YE	*Yersinia enterolitica*	15	[2]	1.57	37.0
LM	*Listeria monocytogenes*	10	[10]	1.52	37.0
EC	*Eschericia coli* NC3	12	[14]	1.90	39.0
BC	*Bacillus coagulans*	14	[18]	0.94	53.9
BS238	*Bacillus stearothermophilus* 238	11	[24]	1.76	65.0
BS	*Bacillus stearothermophilus*	12	[18]	2.84	65.8
TA	*Thermus aquaticus*	12	[18]	0.48	70.2

Table 2 Estimation of the minimum temperature of growth and the power for the selected organisms

Code	T_{min} (°C)	α	r^2
Ps	–5.8 (–7.3;–4.2)	1.77 (1.60;1.94)	0.996
Ac	4.4 (3.2;5.6)	1.21 (1.09;1.33)	0.994
Ae	10.1 (7.8;12.5)	1.18 (0.96;1.40)	0.990
CB	0.0 (–1.5;1.6)	2.21 (1.93;2.51)	0.996
LP	4.5 (4.0;5.0)	1.67 (1.58;1.76)	0.972
YE	–3.8 (–5.1;–2.4)	1.57 (1.38;1.75)	0.988
LM	1.7 (0.5;3.0)	1.55 (1.31;1.78)	0.992
EC	6.5 (4.3;8.6)	1.68 (1.46;1.91)	0.997
BC	26.2 (22.7;29.7)	0.76 (0.38;1.14)	0.824
BS238	38.3 (37.4;39.2)	0.91 (0.78;1.03)	0.993
BS	43.3 (41.8;45.1)	0.68 (0.49;0.88)	0.958
TA	28.1 (3.7;52.5)	2.03 (0.33;3.72)	0.944

is 40°C [22] and 0–5°C [3] respectively. Due to the lack of available data exhibiting low dimensionless growth rate, the upper part of the curve only could be fitted. T_{min} is underestimated for *T. aquaticus*. In contrast, T_{min} is overestimated for *Aeromonas* 4.29. In order to avoid these erroneous estimations of T_{min}, dimensionless growth rate data from 0.1 to 0.2 are required. Due to the shape of the curve μ *vs* temperature, a poor quality of fit is noticed for *B. coagulans* as described in Table 2 by the low regression coefficient. Although, this kind of shape is common to *Bacillus* spp, this shape is particularly pronounced with *B. coagulans*; the growth rate is almost constant at μ_{opt} in the range of 35–60°C. In such an extreme case, the model is not suitable. According to our results, *Clostridium botulinum* type B should be capable of growing at temperatures as low as freezing point. At present, nobody has reported such a psychrotrophic behavior. The data were taken from Graham and Lund [11] who reported that in several experiments, a decrease rather than an increase in numbers occurred at 4°C. Ohye and Scott [21] reported growth at 5°C with a doubling time of 43 h. These results suggest that the result of the only experiment where growth was calculated at 4°C should have been rejected or at least averaged with the other experiments where no growth was detectable.

Two of the 15 data for *Y. enterolitica* exhibit μ_{dim} less than equal to 0.12, leading to an erroneous estimation of the minimum temperature. Should these data be omitted, the estimated minimum temperature of growth increases up to $-1.0°C \pm 1.8°C$, whereas the estimated power decreases down to 1.34 ± 0.21. More generally, an underestimation of T_{min} results in an overestimation of the power and *vice versa*. Therefore, it is necessary to check out for the consistency of the minimum temperature of growth estimates prior to comparing the organisms on the basis of the value of α.

Y. enterolitica serotype 08, is capable of growing at temperatures as low as –1°C [1]. This organism is pre-incubated at the same temperature as the cultivation temperature. Therefore during the pre-incubation period *Y. enterolitica* is acclimated to grow at low temperatures. In contrast, *Clostridium botulinum* was grown at 12°C for tests at 12°C or lower, at 30°C otherwise. A pre-incubation temperature of 12°C is maybe not low enough to ensure cultivation at 4°C, the temperature at which *C. botulinum* is reported to grow normally [13]. These results highlight the influence of pre-incubation on microbial physiology. When compared to the literature [3], no discrepancy was noticed between the estimation and the reported minimum temperature for the growth of the other organisms.

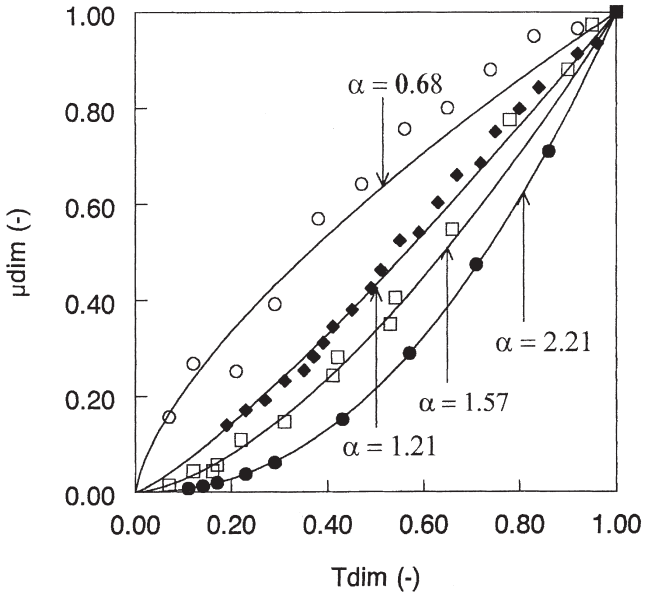

Figure 1 Dimensionless plots, growth rate *vs* temperature for *Bacillus stearothermophilus* (○), *Clostridium botulinum* type B (●), *Yersinia enterolitica* (□), *Acinetobacter* (◆) and power law curves.

It is worth noting that all thermophiles are characterized by low values of α; *T. aquaticus* was not considered for the reasons explained above. These values are less than one, thus exhibiting a convex shape of the curve μ_{dim} *vs* T_{dim}. The power law curve fitting the data of *B. stearothermophilus* is shown in Figure 1. A low value of α is also a characteristic of a non-thermophilic bacillus. The 95% confidence intervals are $\alpha = 1.15 \pm 0.55$, $\alpha = 0.85 \pm 0.41$, for *B. megaterium* and *B. subtilis* respectively; the data were taken from Mohr and Krawiec [18]. The other organisms, mesophiles and psychrotrophs, are characterized by higher values of α, thus exhibiting a concave shape of the curve μ_{dim} *vs* T_{dim}. The power law curves are shown in Figure 1 for *C. botulinum*, *Yersinia enterolitica* and *Acinetobacter* which exhibit different values for α. It is pointed out that all of the organisms considered in this paper, except *C. botulinum*, exhibit values of α significantly different from two.

Conclusions

The dimensionless analysis allows the comparison between organisms characterized by different positions in the temperature spectrum. By using a normalized variable for the growth rate, μ/μ_{opt}, thermophiles with high optimal growth rates can be compared with other organisms. The determination procedure for the growth rate can lead to different results, especially in the case of confusion between the growth rate and the specific growth rate. It has been assumed in this paper that the dimensionless growth rate is independent from the calculation procedure utilized, but this should be verified. Using a dimensionless temperature

is very convenient, not only because thermophiles can be compared to other organisms, but also because any unit for the temperature can be used. The organisms have been grown on different, but adequate media. For example both *L. monocytogenes* and *Y. enterolitica* were grown on brain heart infusion. Therefore only temperature constrained the growth rate. It was assumed from the operating conditions reported in the literature that nothing such as the agitation or the mixing conditions limited growth, apart from the temperature. In contrast, when the medium composition is not optimal, the minimum temperature changes. The influence of the media composition on the power, α, should also be examined.

The model is based on biological parameters. Therefore, the determination of the parameters obtained at the optimal temperature can be made experimentally. The estimation of the minimum temperature can be compared to tables or literature data. For example, the minimum temperature estimation for *T. aquaticus* has been rejected on this basis. An accurate determination of T_{min} can be obtained provided growth rate data at low temperatures are available. The dimensionless variables have been obtained on the basis of a linear transformation. Therefore the model cannot represent the S-shaped curve of the growth rate *vs* temperature. When the inflection point is close to the optimum temperature, the discrepancy between the experimental results and the model is not noticeable as suggested by regression coefficients close to one. The model is not suitable, for example, for *Bacillus coagulans* which presents a wide range of temperatures at which the growth rate almost equals μ_{opt}, thus leading to a low coefficient correlation. Thermophiles are characterized by a power value less than mesophiles and psychrotrophs which are described by higher values. Despite the heterogeneity of the results (number of data, position of the data in the temperature spectrum, confidence in the growth rates determined at low temperatures), some mesophiles and psychrotrophs are characterized by significantly different values of power. In most cases, the power is significantly different from two. These results should lead to better predictions in predictive microbiology.

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