Food Microbiology, 2000, **17**, 597–604 doi:10.1006/fmic.2000.0355
Available online at http://www.idealibrary.com on IDEAL®

ORIGINAL ARTICLE

Influence of the modelling approach on the estimation of the minimum temperature for growth in Bělehrádek-type models

Philippe Dantigny[1]* and Paul Molin[2]

This paper compares three different approaches for describing the growth rate dependence on sub-optimal temperatures {e.g., the square-root model described by Ratkowsky et al. (1982. J. Bacteriol. **154***, 1222–1226):* $\sqrt{\mu} = \mathrm{b}.(\mathrm{T} - \mathrm{T}_{\mathrm{min1}})$ *or* $\mu = \mathrm{b}^2.(\mathrm{T} - \mathrm{T}_{\mathrm{min1}})^2$*, the model originally found by Bělehrádek (1926a.* Nature **118***, 117–118):* $\mu = a.(\mathrm{T} - \mathrm{T}_0)^{\alpha}$ *and a dimensionless analysis described previously, (Dantigny 1998.* J. Ind. Microbiol. Biotechnol. **21***, 215–218.):*

$$\mu_{\mathrm{dim}} = \left[\frac{\mu}{\mu_{\mathrm{opt}}}\right] = \left[\frac{\mathrm{T} - \mathrm{T}_{\mathrm{min}}}{\mathrm{T}_{\mathrm{opt}} - \mathrm{T}_{\mathrm{min}}}\right]^{\alpha} = [\mathrm{T}_{\mathrm{dim}}]^{\alpha}\}.$$

Data sets, growth rate vs temperature, have been taken from the literature for various organisms (e.g. Lactobacillus plantarum, Yersinia enterolitica *and* Acinetobacter*).*

Firstly, this paper analyses the effect of using dimensionless (e.g. $\mathrm{T}_{\mathrm{dim}}$ *and* μ_{dim}*) or natural variables (e.g.* T *and* μ*) on the estimation of the minimum temperature for growth (e.g.* T_0 *and* $\mathrm{T}_{\mathrm{min}}$*) and the* α *value. Secondly, the Bělehrádek model is compared to the square-root model by using the natural variables. It has been demonstrated that the use of the square-root model leads to an under-estimation of the minimum temperature for growth when the* α*-value is significantly less than 2. In such a case, it has been highlighted that the dimensionless approach provides a closer estimation of the experimental minimum temperature for growth than the square-root model.*

Introduction

Amongst the environmental factors that affect microbial growth, temperature is an important parameter to consider for process transformation 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. The Arrhenius law was originally proposed to describe the temperature dependence of the specific growth rate during the exponential growth phase and has been used by several authors (Hanus and Morita 1968, Herendeen et al. 1979, Ingraham 1958, O'Donovan et al. 1965, Shaw 1967). Other mechanistic approaches based on the Arrhenius law have been developed (Sharpe and DeMichele 1977, Schoolfield et al. 1981). Unfortunately, the original relationship fits the data poorly, as curves, rather than straight lines, are obtained when the natural logarithm of the growth rate constant is plotted against the reciprocal of

*Corresponding author. Fax: 33 (0)3 80 39 66 40. E-mail: phdant@u-bourgogne.fr

Received: 14 December 1999

[1]Laboratoire de Microbiologie, UMR INRA 1082, ENSBANA, 1, Esplanade Erasme, F-21000, Dijon, France
[2]Laboratoire de Génie des Procédés Alimentaires et Biotechnologiques, Dijon, France

0740-0020/00/060597 + 08 $35.00/0

the temperature. Mohr and Krawiec, (1980) claimed two distinct slopes for some mesophiles and thermophiles, but these observations were rejected by Ratkowsky et al. (1982) who suggested empirical square-root models. These models are based upon the observation of Ohta and Hirahara (1977) that the square-root of nucleotide degradation in carp muscle is related to temperature. The square-root model developed by Ratkowsky et al. (1982) is valid at sub-optimal temperatures only. Subsequently, it was found (Ross, 1987) that the square-root model: $\sqrt{\mu} = b.(T - T_{\min 1})$ or $\mu = b^2.\ (T - T_{\min 1})^2$ is a particular case of the Bělehrádek (1926a, 1926b) model based upon a power function law: $\mu = a.(T - T_0)^{\alpha}$. It was found that the α-value is greatly dependent on the type of the biological reaction, ranges from 0·90 to 2·14 (amœboid movement); from 1·16 to 4·10 (embryonic development) and from 1·06 to 3·00 (heart-beat). McMeekin et al. (1987) demonstrated that the power value of 2 can be used for *Staphylococcus xylosus*. By extension, this value has been widely used in predictive microbiology for describing the growth of foodborne pathogens.

More recently (Dantigny 1998) a dimensionless approach, based on biological parameters (e.g. $\mu_{\text{opt}}, T_{\text{opt}}$ and $T_{\min}$), has been described for comparing the growth rate dependence on sub-optimal temperatures of some thermophilic, mesophilic and psychrotrophic organisms. By implementing the following normalized variables; $\mu_{\dim} = \mu/\mu_{\text{opt}}$ and $T_{\dim} = [T - T_{\min}]/\ [T_{\text{opt}} - T_{\min}]$, within a power function law: $[\mu_{\dim}] = [T_{\dim}]^{\alpha}$, it has been demonstrated that the thermophiles considered in this study are characterized by α-values less than 1, whereas the other mesophiles and psychrotrophs can be described by α-values ranging from 1·18 to 2·21. These results suggest that some organisms are characterized by a power different from 2, which is the value widely used in predictive microbiology for describing the growth rate dependence on the temperature. Although, the influence of using dimensionless variables and setting the optimal parameters (e.g., T_{opt} and μ_{opt}) on the estimation of the power and the minimum temperature for growth was not stated.

Therefore, it is the main objective of the paper to analyse through literature data (e.g., growth rate vs temperature) already fitted by the square-root model, (i) the influence of the variables (dimensionless or natural) implemented within the Bělehrádek model on the estimation of α and the minimum temperature for growth and (ii) the consequences of using the square-root model for an organism characterized by a power-value different from 2.

Materials and Methods

Graphs have been taken from the literature (e.g. *Lactobacillus plantarum* Zwietering et al. 1994, *Yersinia enterolitica*: Alber and Schaffner 1993, *Acinetobacter* 4·41: Ratkowsky et al. 1983) and have been scanned. Then individual points have been digitized by means of software (UnGraph 4·0, BioSoft, Cambridge, UK). In order to stabilize the variance of the growth rate, a logarithmic transformation has been used (Alber and Schaffner 1992, Zwietering et al. 1994, Schaffner 1998) for all model equations. The model parameters have been estimated by a non-linear regression software based upon the Levenberg-Marquardt Algorithm, (SlideWrite 4·1, Advanced Graphics Software, Inc., Carlsbad, California, 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.

Models

Square-root model

$$\mu = b^2.[T - T_{\min 1}]^2 \tag{1}$$

μ (h^{-1}) is the specific growth rate evaluated during the exponential growth phase, b ($^{\circ}C^{-1}.h^{-0.5}$) is a parameter without any biological meaning, $T_{\min 1}$ ($^{\circ}C$) is the minimum temperature for growth. After data trans-formation, Eqn (1) becomes:

$$Ln(\mu) = 2.Ln(b) + 2.Ln(T - T_{\min 1}) \tag{2}$$

Bělehrádek model

Natural variables

$$\mu = a.[T - T_0]^{\alpha} \quad (3)$$

a (h^{-1}) is a constant, T_0 ($^{\circ}C$) is the biological zero, α is a parameter to be estimated which has probably a wide biological interest (Bělehrádek 1926b). It should be noted here that, in order to avoid any confusion between models nomenclature, the original notations of the Bělehrádek model have been altered.
After data transformations, Eqn (3) becomes:

$$Ln(\mu) = Ln(a) + \alpha.Ln(T - T_0) \quad (4)$$

Dimensionless variables

$$\mu_{dim} = \left[\frac{\mu}{\mu_{opt}}\right] = \left[\frac{T - T_{min}}{T_{opt} - T_{min}}\right]^{\alpha} = [T_{dim}]^{\alpha} \quad (5)$$

T_{opt} is the temperature at which the specific growth rate, μ_{opt}, is maximal. 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 temperature. Usually the units are (h^{-1}) and ($^{\circ}C$) for μ and T respectively, although the use of dimensionless variables (e.g. with no units): μ_{dim} and T_{dim}, allows any other unit to be used. For example, temperature unit can be Celsius, Kelvin, or even Farenheit, In addition, the dimensionless variables are normalized, they are varying in the range [0,1]. After data transformation, Eqn (5) becomes:

$$Ln(\mu) = Ln(\mu_{opt}) + \alpha[Ln(T - T_{min}) - Ln(T_{opt} - T_{min})] \quad (6)$$

Equation (5) can be rewritten as follows:

$$\mu = \frac{\mu_{opt}}{[T_{opt} - T_{min}]^{\alpha}}[T - T_{min}]^{\alpha} \quad (7)$$

Assuming that T_{min} and T_0 are both representing the minimum temperature for growth, Eqn (7) can be seen as a particular case of the Bělehrádek equation in which $a = \mu_{opt}/[T_{opt} - T_{min}]^{\alpha}$.

Results

Influence of the variables

The results of the simulations are reported in Table 1. By using the dimensionless variables,

Table 1. Parameter values and model estimations for *Lb. plantarum, Yersinia enterolitica* and *Acinetobacter* from the data: growth rate vs temperature

Organism	Model	Estimates	95% confidence interval	Fixed parameters	r^2
	Square-root model	$b = 0{\cdot}0375$, $T_{min1} = 3{\cdot}32$	0·0363 ; 0·0387, 3·02 ; 3·61	None	0·983
Lb. plantarum	Belehrádĕk model	$a = 0{\cdot}00174$, $T_0 = 3{\cdot}57$, $\alpha = 1{\cdot}94$	0·00079 ; 0·00270, 2·87 ; 4·27, 1·77 ; 2·10	None	0·983
	Dimensionless approach	$T_{min} = 4{\cdot}51$, $\alpha = 1{\cdot}67$	4·04 ; 4·99, 1·58 ; 1·76	$T_{opt} = 37{\cdot}0$, $\mu_{opt} = 1{\cdot}27$	0·972
	Square-root model	$b = 0{\cdot}0315$, $T_{min1} = -6.29$	0·0289 ; 0·0340, −7·27 ; −5·31	None	0·986
Yerisinia enterolitica	Belehrádĕk model	$a = 0{\cdot}00355$, $T_0 = -4{\cdot}14$, $\alpha = 1{\cdot}66$	−0·00034 ; 0·00745, −5·95 ; −2·33, 1·35 ; 1·96	None	0·989
	Dimensionless approach	$T_{min} = -3{\cdot}77$, $\alpha = 1{\cdot}57$	−5·12 ; −2·43, 1·38 ; 1·75	$T_{opt} = 37{\cdot}0$, $\mu_{opt} = 1{\cdot}57$	0·988
	Square-root model	$b = 0{\cdot}0315$, $T_{min1} = -2{\cdot}36$	0·0300 ; 0·0330, −3·25 ; −1·48	None	0·990
Acinetobacter	Belehrádĕk model	$a = 0{\cdot}0116$, $T_0 = 3{\cdot}47$, $\alpha = 1{\cdot}35$	0·0032 ; 0·199, 1·72 ; 5·22, 1·14 ; 1·55	None	0·995
	Dimensionless approach	$T_{min} = 4{\cdot}40$, $\alpha = 1{\cdot}21$	3·17 ; 5·64, 1·09 ; 1·33	$T_{opt} = 29{\cdot}0$, $\mu_{opt} = 0{\cdot}87$	0·994

the α-values are significantly different from 2 in all cases, when compared to the square-root model. Although the square-root model does not utilize dimensionless variables, but the natural ones. Therefore, the results for the Bělehrádek model obtained through using the dimensionless approach and the natural variables should be compared first. For *Lb. plantarum*, the α-value determined by means of the dimensionless variables, 1·67, differs significantly from $\alpha = 1{\cdot}94$ found with the natural variables. In contrast, the use of either the dimensionless or the natural variables in Bělehrádek model does not affect significantly the α value for *Yersinia enterolitica* and *Acinetobacter*. Moreover, these organisms exhibit α values significantly different from 2.

For the Bělehrádek model, it is shown on Table 1 by comparing T_0 and $T_{\min}$ for all the organisms, that the choice of the variable (natural or dimensionless) has no significant influence on the estimation of the minimum temperature for growth. Although it should be stated that fixing the optimal parameters is of paramount importance for *Lb. plantarum*. The curve growth rate vs temperature as shown in Fig. 1 exhibits a pronounced S-shape, with an inflexion point at about 27°C, thus preventing any of the equation based on a power law to fit accurately all the experimental data. When the optimal parameters are not set, a great discrepancy occurs at temperatures close to T_{opt}. For example the square-root model predicts a growth rate of 1·59 h^{-1} at the optimal temperature of 37°C, whereas μ_{opt} is about 1·27 h^{-1} experimentally. In contrast, the use of the dimensionless approach with setting the optimal parameters prevents from fitting the data in the range 20–30°C, thus leading to a low regression coefficient of 0·972. The low regression coefficient obtained for *Lb. plantarum* is due to setting the parameters T_{opt} and μ_{opt} at fixed values and to the S-shaped curve μ vs T. In the case of *Y. enterolitica* and *Acinetobacter*, the S-shape is not so pronounced (see on Figs 2 and 3), there is no penalty in using fixed parameters as suggested by the regression coefficients reported in Table 1.

Influence of the model

In contrast it is shown in Table 1 that the best fit can be obtained through using the natural variables in the Bělehrádek equation. In fact, better regression coefficients are obtained when the power of the function is a variable to be estimated. Although, two parameters are to

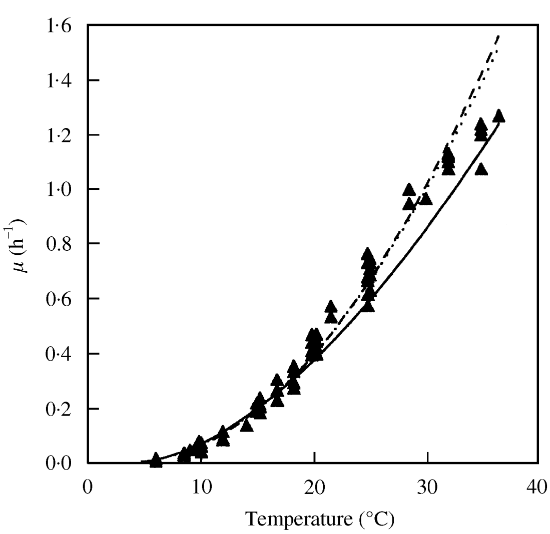

Figure 1. Comparison between the square-root model (- - -) the Bělehrádek model (. . .) and the dimensionless approach (—) for fitting the *Lb. plantarum* data (▲) growth rate vs temperature.

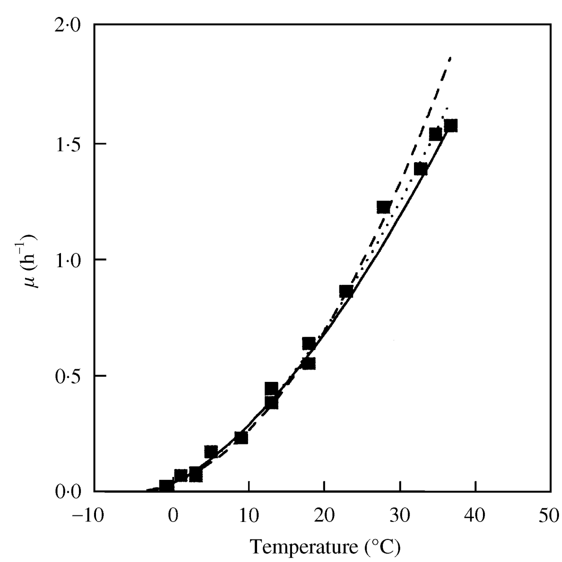

Figure 2. Comparison between the square-root model (- - -) the Bělehrádek model (. . .) and the dimensionless approach (—) for fitting the *Y. enterolitica* data (■) growth rate vs temperature.

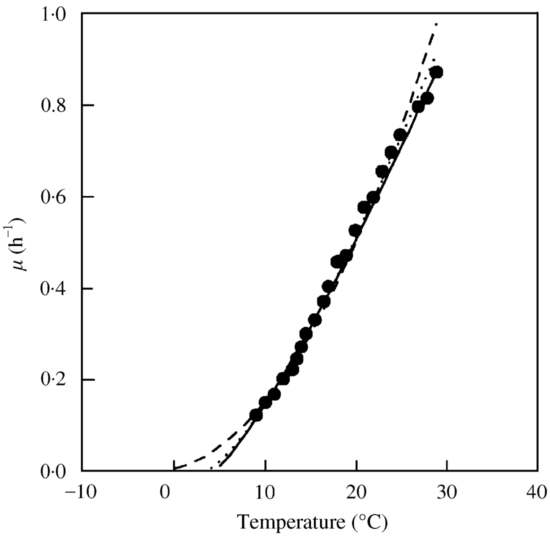

Figure 3. Comparison between the square-root model (- - -) the Bělehrádek model (. . .) and the dimensionless approach (—) for fitting the *Acinetobacter* data (●) growth rate vs temperature.

be estimated when using the square-root model as compared to three parameters when using the Bělehrádek equation. Therefore, no fair comparison can be made, the advantages and the drawbacks of each model will be discussed in the next section.

Lactobacillus plantarum is characterized by estimates which are not significantly dependent from the model. In such a case, the α-value almost equals 2, the organism can be described by the square-root model. Accordingly, there is no great difference between $T_{\min 1}$ and T_0.

Yersinia enterolitica can be considered as an intermediate case. The α-value is significantly different from 2, but the estimation of the minimum for growth does not significantly depend from the model.

More interestingly, *Acinetobacter* exhibits differences between both the estimations of the power and the minimum temperature for growth. The estimation, $T_{\min 1} = -2{\cdot}36°C$, is below the freezing point with the square-root model. *Acinetobacter* is a psychrotrophic Gram-negative rod-shaped bacteria capable of growing at low temperatures ranging from 3 to 7°C (Larpent 1996). Pin and Baranyi (1998) have reported that a strain isolated from refrigerated lamb carcass meat developed at 2°C, although at a very low growth rate. But, to our knowledge, nobody has reported that any strain of *Acinetobacter* is capable of growing at negative temperatures. Therefore the estimations of the minimum temperature for growth, $T_0 = 3{\cdot}47°C$ and $T_{\min} = 4{\cdot}40°C$, obtained with the Bělehrádek equation and the dimensionless approach respectively, are closer to the experimental minimum temperature for growth than $T_{\min 1}$. In addition, it is highlighted that the power is far different from 2. As suggested by a comparison between the regression coefficients reported in Table 1, the square-root model performs less satisfactorily than the Bělehrádek model. It is shown in Fig. 3, that the minimum temperature for growth, $T_{\min 1}$, is under estimated while using the square-root model. This is due to the use of 2 for the power in the square-root equation. It is demonstrated in the appendix section through a comparison between the square-root model and the Bělehrádek model that a power α less than 2 leads to T_0 greater than $T_{\min 1}$ under certain conditions.

Discussion

There is more interest in sub-optimal temperatures in the fields of food preservation, for goods are stored at room temperature or in cold rooms. In such cases, it is very unlikely that the temperature exceeds the optimal one. Accordingly, in this paper the influence of temperature throughout the entire biokinetic range was not examined. Although, most models used in predictive microbiology are valid from $T_{\min}$ to $T_{\max}$ (maximum temperature for growth). Aside from a wider validity range, these models allows a better accuracy at temperatures close to T_{opt}. The S-shaped curve, growth rate vs temperature, exhibits an inflexion point. Therefore, the power law function cannot fit the data at temperatures close to the optimum. The square-root model has extent its use up to $T_{\max}$ (Ratkowsky et al. 1983) who suggested the relationship: $\mu = \{b.(T - T_{\min 2}).(1 - \exp(c[T - T_{\max 2}]))\}^2$ latter altered by Zwietering et al. (1994) to: $\mu = \{b.(T - T_{\min 3})\}^2.(1 - \exp(c[T - T_{\max 3}]))$: It should be pointed out that, aside from extending the validity range of the model, the factor

$(1 - \exp(c[T - T_{\max}]))$ allows an inflexion point to be exhibited through the use of the square-root model. Thus, a better prediction under optimal conditions can be obtained through using models valid throughout the entire biokinetic range.

A better fit can be obtained by setting the optimal parameters μ_{opt} and T_{opt} in the Bělehrádek model. Although, for organisms characterized by a pronounced S-shaped curve μ vs T (such as *Lb. plantarum*) the dimensionless approach leads to a lower regression coefficient than when the natural variables (e.g. μ and T) are used. It has been shown previously (Dantigny 1998) that a very pronounced S-shaped curve μ vs T prevents one from using the dimensionless approach. The use of dimensionless growth rate and temperature allows the comparison between organisms classified into distinct classes by their position in the temperature spectrum, the use of different definitions for the growth rate (Demetz and Dantigny 2000) and units for the temperature (Dantigny 1997), the comparison between environmental factors, temperature and water activity, for the fungal development of *Penicillium chrysogenum*, (Dantigny et al. 2000). Although, to become even more attractive, the dimensionless approach should be applied to model equations valid throughout the entire biokinetic range. In such a case, there is probably no penalty in determining experimentally the optimum parameters. For example, the cardinal temperature model with inflection developed by Rosso et al. (1993) provides an estima tion of the optimum parameters. In general the precision of the estimates are about $+/-2°$C and $+/-10\%$ for T_{opt} and μ_{opt} respectively. A similar accuracy can be attainable by direct reading.

The square-root model is a particular case of the Bělehrádek equation, but sometimes the square-root model is called Bělehrádek-type model. There is a great difference in both the approaches. The square-root model considers that the curvature of the representation growth rate vs temperature is fixed and does not depend on the micro-organism. In contrast, Bělehrádek considered that the power α has probably a wider biological interest. In fact, it has been demonstrated that organisms are characterized by different α-values. In addition, it has been highlighted in this paper that the use of the square-root model to describe the influence of temperature on the growth rate of an organism characterized by a α-value less than 2 leads to an under estimation of T_{min1} which is recognised as one of the weakness of the square-root model (Ross 1993). Therefore, it is suggested for a better prediction in food microbiology, to check out whether another exponent than 2 should be used, especially when a great discrepancy between the estimated and the observed minimum temperature for the growth of an organism is pointed out.

References

Alber, S. A. and Schaffner, D. W. (1992) Evaluation of data transformations used with the square root and Schoolfield models for predicting bacterial growth rate. *Appl. Environ. Microbiol.* **58,** 3337–3342.

Alber, S. A. and Schaffner, D. W. (1993) New modified square root and Schoolfield models for predicting bacterial growth rate as a function of temperature. *J. Ind. Microbiol.* **12,** 206–210.

Bělehrádek, J. (1926a) Influence of temperature on biological processes. *Nature* **118,** 117–118.

Bělehrádek, J. (1926b) Protoplasmic viscosity as determined by a temperature coefficient of biological reactions. *Nature* **118,** 478–480.

Dantigny, P. (1997) A dimensionless Bělehrádek-type model for sub-optimal temperatures based on biological parameters. In *Proceedings of predictive microbiology applied to chilled food conservations.* (Eds) C. M. Bourgeois and T. A. Roberts pp. 72–78, Brussels. Office for Official Publications of the European Communities.

Dantigny, P. (1998) Dimensionless analysis of the microbial growth rate dependence on sub-optimal temperatures. *J. Ind. Microbiol. Biotechnol* **21,** 215–218.

Dantigny, P., Sautour, M., Bensoussan, M. and Divies, C. (2000) Use of dimensionless correlations: from biochemical engineering to predictive microbiology. In *Proceedings of FOODSIM'2000*, Nantes, France, June 26–27, 2000. (Ed. Dithiet) pp. 146–150. Ghent, Society for Computer Simulation.

Demetz, D. and Dantigny, P. (2000) Influence of the growth rate calculation on the relationship between the growth rate and temperature. *Lett. Appl. Microbiol.* **30,** 272–276.

Hanus, F. J. and Morita, J. Y. (1968) Significance of the temperature characteristic of growth. *J. Bacteriol.* **95,** 736–737.

Herendeen, S. L., VanBogelen, R. A. and Neidhardt, F.C. (1979) Levels of major proteins of *Escherichia coli* during growth at different temperatures. *J. Bacteriol.* **139,** 185–194.
Ingraham, J. L. (1958) Growth of psychrophilic bacteria. *J. Bacteriol* **76,** 75–80.
Larpent, J. P., (1996) Laits et produits laitiers non fermentés. In *Microbiologie alimentaire.* Tome 1. (Eds) C. M. Bourgeois, J. F. Mescle and J. Zucca, pp. 272–294. TEC et DOC Lavoisier, Paris, France.
McMeekin, T. A., Chandler, R. E., Doe, P. E., Garland, C. D., Olley, J., Putro, S. and Ratkowsky, D. A. (1987) Model for combined effect of temperature and salt concentration/water activity on the growth rate of *Staphylococcus xylosus. J. Appl. Bacteriol.* **62,** 543–550.
Mohr, P.W. and Krawiec, S. (1980) Temperature characterisitics and Arrhenius plots for nominal psychrophiles, mesophiles and thermophiles. *J. Gen. Microbiol.* **121,** 311–317.
O'Donovan, G. A., Kearne, C. L. and Ingraham, J. L. (1965) Mutants of *Escherichia coli* with high minimal temperatures of growth. *J. Bacteriol* **90,** 611–616.
Ohta, F. and Hirahara, T. (1977) Rate of degradation of nucleotides in cool-stored carp muscle. *Memo. Fac. Fish. Kagoshima. Univ.* **26,** 97–102.
Pin, C. and Baranyi, J. (1998) Predictive models as means to quantify the interactions of spoilage organisms. *Int. J. Food. Microbiol.* **41,** 59–72.
Ratkowsky, D. A., Olley, J., McMeekin, T. A. and Ball, A. (1982) Relationship between temperature and growth rate of bacterial cultures. *J. Bacteriol.* **149,** 1–5.
Ratkowsky, D. A., Lowry, R. K., McMeekin, T. A., Stokes, A. N. and Chandler, R. E. (1983) Model for bacterial culture growth rate throughout the entire biokinetic temperature range. *J. Bacteriol.* **154,** 1222–1226.
Ross, T. (1987) Bělehrádek temperature functions and growth of organisms. *CSIRO-DSIR Joint Workshop on Seafood Processing, Nelson, New Zealand, April 1986.* Occasional Paper 18, CSIRO Tasmanian Regional Laboratory, Hobart, Australia, pp. 16.
Ross, T. (1993) Bělehrádek-type models. *J. Ind. Microbiol.* **12,** 180–189.
Rosso, L., Lobry, J. R. and Flandrois, J. P. (1993) An unexpected correlation between cardinal temperatures of microbial growth highlighted by a new model. *J. theor. Biol.* **162,** 447–463.
Schaffner, D. W. (1998) Predictive food microbiology Gedanken experiment: why do microbial growth data require a transformation. *Food. Microbiol.* **15,** 185–189.
Schoolfield, R. M., Sharpe, P. J. H. and Magnuson, C. E. (1981) Non-linear regression of biological temperature-dependent rate models based on absolute reaction rate theory. *J. Theor. Biol.* **88,** 719–731.
Sharpe, P. J. H. and DeMichele, D.W. (1977) Reaction kinetics of poikilotherm development *J. Theor. Biol.* **64,** 649–670.
Shaw, M. K. (1967) Effect of abrupt temperature shift on the growth of mesophilic and psychrophilic yeasts. *J. Bacteriol.* **93,** 1332–1336.
Zwietering, M. H., Cuppers, H. G. A. M., de Wit, J. C. and van't Riet, K. (1994) Evaluation of data transformations and validation of a model for the effect of temperature on bacterial growth. *Appl. Environ. Microbiol.* **60,** 195–203.

Appendix

Let's consider the number of intercepts between the square-root model:

$$\mu = f(T) = b^2.(T - T_{\min 1})^2 \qquad \text{(A1)}$$

and the Bělehrádek equation:

$$\mu = g(T) = a.(T - T_0)^\alpha \qquad \text{(A2)}$$

Both the functions described by Eqns (A1) and (A2) exhibit convex shapes if α is greater than 1, which is the case in this paper for organisms herein considered.

Let's define: $h(T) = f(T) - g(T)$. By studying the variations of $h(T)$, $h'(T)$ and $h''(T)$ between T_0 and T, it can be easily demonstrated (not shown) that the two convex functions, as above described, exhibit no more than two intercepts if α is less than 2.

No intercept

Both the functions are fitting the same experimental data. For the curvatures are different, (it is assumed that α is different from 2), no intercept between both the functions should have led to a bias fitting. Therefore there is at least one intercept.

One intercept:

For $T \to +\infty$, $b^2.(T - T_{\min 1})^2 > a.(T - T_0)^\alpha$: the growth rate calculated through the square-root model is over the growth rate calculated by means of the Bělehrádek function.

Let's define the intercept as $(T_{\text{int}}, \mu_{\text{int}})$, eq (3) makes sense if T_{int} is greater than equal to T_0, only.

For $T > T_{\text{int}}$, $b^2.\ (T - T_{\min 1})^2 > a.(T - T_0)^\alpha$, and for $T < T_{\text{int}}$, $b^2.(T - T_{\min 1})^2 < a.(T - T_0)^\alpha$

Therefore, for $T < T_{\mathrm{int}}$, the square-root curve is under the Bělehrádek one.

Accordingly, $T_0 < T_{\mathrm{min1}}$.

Let's calculate the derivative functions, for $T = T_{\mathrm{min1}}$, and for $T = T_0$ for the square-root model and the Bělehrádek function respectively.

$$\left[\frac{df}{dT}\right]_{T=T_{\mathrm{min1}}} = 2.b^2 \quad \text{(A3)}$$

$$\left[\frac{dg}{dT}\right]_{T=T_0} = \alpha.a. \quad \text{(A4)}$$

Calculating the ratio (A3)/(A4):

$$\frac{\left[\frac{df}{dT}\right]_{T=T_{\mathrm{min1}}}}{\left[\frac{dg}{dT}\right]_{T=T_0}} = \frac{2}{a}\frac{b^2}{\alpha} \quad \text{(A5)}$$

All the organisms herein considered are characterised by the ratio $2b^2/a\alpha$ less than one: *Lb. plantarum*, 0·833, *Y. enterolitica*, 0·337, *Acinetobacter*, 0·127. At minimum temperature for growth, the derivative at $T = T_0$, $g'(T_0)$ is greater than the derivative at $T = T_{\mathrm{min1}}$, $f'(T_{\mathrm{min1}})$.

Both $f(T)$ and $g(T)$ are convex, strictly monotonous functions, therefore for a given temperature, the derivative function $g'(T)$ is greater than $f'(T)$. In such a case, no intercept can be found if $T_0 < T_{\mathrm{min1}}$.

Two intercepts:

Thus, the only solution lays in two intercepts as illustrated in Fig. A1 for *Acinetobacter*. The assumption of two intercepts is also confirmed for *Lb. plantarum* and *Yersinia enterolitica*.

There is a first intercept at T_{int1} for which $f(T_{\mathrm{int1}}) = g(T_{\mathrm{int1}})$. It is clearly shown, that for $T > T_{\mathrm{int1}}$; $f(T) > g(T)$; the square-root curve is over the Bělehrádek one.

There is a second intercept at T_{int2} for which $f(T_{\mathrm{int2}}) = g(T_{\mathrm{int2}})$. For definition purpose, we also have: $T_{\mathrm{int2}} > T_0$ and $T_{\mathrm{int2}} > T_{\mathrm{min1}}$. For $T_{\mathrm{int2}} < T < T_{\mathrm{int1}}$, $f(T) < g(T)$; the square-root curve is under the Bělehrádek one.

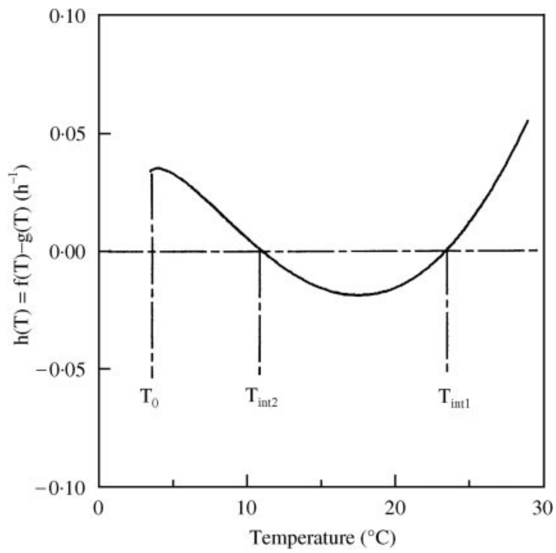

Figure A1. Difference between the estimation of the growth rate given by the square-root model: $f(T)$ in eq (A1) and the estimation of the growth rate given the Bělehrádek model: $g(T)$ in eq (A2) for *Acinetobacter*.

Eventually, for $T < T_{\mathrm{int2}}$; the square-root curve is over the Bělehrádek one.

From T_{int2} with decreasing temperature, there is no other intercept until the intercept with the X-axis, which occurs first at $T = T_0$ for the lower curve (e.g., Bělehrádek equation) and then for $T = T_{\mathrm{min1}}$ for the square-root model (not shown on Fig. A1, for Eq (A2) is not defined for $T < T_0$).

More generally, providing that $2b^2/a\alpha$ is less than one, the use of the square-root model whereas a value $\alpha < 2$ should be used in Bělehrádek model leads to an underestimation of the minimum temperature for growth: $T_0 > T_{\mathrm{min1}}$.