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An Unexpected Correlation between Cardinal Temperatures of Microbial Growth Highlighted by a New Model

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A new model for the prediction of microbial-specific growth rate as a function of temperature is presented. The four parameters of this model are the three cardinal temperatures (T_{max}, T_{min} and T_{opt}) and the specific growth rate at the optimum temperature (μ_{opt}). A comparison with three other models was made on the basis of several criteria (simplicity and biological significance of parameters, applicability, quality of fit, minimum structural correlations and ease of determination of parameters). A detailed comparison of a 217-point data set, and an extensive comparison of 47 different data sets show that the new model is better than its competitors. The three cardinal temperatures were found to be independent of μ_{opt}. A very strong and unexpected linear correlation between the cardinal temperatures was observed. The consequences of this biological result are discussed, even though causes remain unknown.

Introduction

Temperature is an important factor in the growth of micro-organisms. This environmental factor is of fundamental interest in taxonomy (definition of thermo-, meso- and psychrophilic groups) and in the investigation of microbial metabolism. Temperature is also of practical importance in the control of bioprocesses in biotechnology, and safe handling of goods, especially in agricultural and food industry.

The fundamental and practical importance of temperature for microbial growth has led to the publication of various models. These models are used to summarize and describe the effect of temperature on the specific growth rate during the exponential phase (μ_{max}). Models are also of interest for obtaining a continuous description by interpolation, and, tentatively, for predicting by extrapolation.

Two different approaches have been used for model building. The first is the mechanistic approach based on the Arrhénius formulation and the hypothesis of the "determining reaction" responsible for variations in growth rate when the temperature changes. Many models are based on this approach (Hinshelwood, 1946; Mohr & Krawiec, 1980; Esener *et al.*, 1981; Schoolfield *et al.*, 1981). The second approach is empirical: the model is constructed from mathematical functions and its suitability to fit to biological data is then tested. This method was chosen by Ratkowsky *et al.* (1982, 1983) with the square-root model, and in the present paper to build a new empirical model, with the aim of comparing it with those previously published.

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Materials and Methods

MODELS

The change in maximum specific growth rate μ_{max} (hr^{-1}) as a function of temperature T (°C) has been described with four different models. These models have been selected because they all have four parameters. This allows for easier comparison, since comparison of non-linear non-nested models with differing numbers of parameters is difficult. Moreover, usual data sets do not allow the estimation of more than four parameters because the number of experimental data are not sufficient.

(i) *Hinshelwood model (H)*

$$\mu_{max} = A_1 e^{-B_1/T} - A_2 e^{-B_2/T}. \quad (1)$$

μ_{max} is the maximum specific growth rate (hr^{-1}); A_1, A_2, B_1 and B_2 are four parameters without biological interpretation and without a direct graphical counterpart when plotting the model. From the Hinshelwood point of view, the parameters could be interpreted thermodynamically.

(ii) *Ratkowsky complete model (RTK2)*

$$\mu_{max} = [b(T - T_{min})(1 - e^{c(T - T_{max})})]^2 \quad (2)$$

b ($°C^{-1}\ hr^{-0.5}$) and c ($°C^{-1}$) were constant parameters, T_{min} was the minimum growth-temperature and T_{max} the maximum growth-temperature. This model (Ratkowsky *et al.*, 1983) is an extended version of a previously published one (Ratkowsky *et al.*, 1982).

(iii) *Zwietering model (ZWT)*

This model proposed by Zwietering *et al.* (1991) was derived from the Ratkowsky's formulation and presented by the authors as an alternate form of the RTK2 model:

$$\mu_{max} = [b(T - T_{min})]^2(1 - e^{c(T - T_{max})}). \quad (3)$$

(iv) *Cardinal temperature model with inflection (CTMI)*

This new model was built empirically as a tool to describe data without the aim of a mechanistic explanation. As the three cardinal temperatures are often used in microbiology (Stanier *et al.*, 1986) to characterize the effect of temperature on growth in an approximative manner, we have chosen to include them explicitly as parameters. Another reason for building this new model was that a preliminary study showed that the popular RTK2 model is badly conditioned which means in practice that its parameters are difficult to estimate. The CTMI model was derived from a previously published one (Lobry *et al.*, 1991) to account for the experimentally observed point

of inflection in the suboptimal range of temperatures:

$$\mu_{max} = \text{Sup}\left[0{\cdot}0, \frac{\mu_{opt}(T-T_{max})(T-T_{min})^2}{(T_{opt}-T_{min})[(T_{opt}-T_{min})(T-T_{opt})-(T_{opt}-T_{max})(T_{opt}+T_{min}-2T)]}\right] \quad (4)$$

where T_{min} (°C) is the temperature below which growth is no longer observed, T_{max} (°C) is the temperature above which no growth occurs, T_{opt} (°C) is the temperature at which the maximum specific growth rate equals its optimal value μ_{opt} (hr^{-1}).

Data

The numerical value of all data sets used here are available on floppy disks (1·4 Mo for the Macintosh). The data sets were taken from the literature.

DATA FOR PRELIMINARY ACCURATE COMPARISON

The *Escherichia coli* data set of Barber (1908) was used to describe and compare the four models. This data set was chosen because it contains an exceptionally large number of points which allows for good accuracy. The maximum growth rate was deduced from the doubling time given in this paper as a function of temperature. Five outliers were rejected and three points illegible because of the poor quality photocopy of this historic paper. When the author gave a range of temperatures instead of an exact value, the arithmetic mean was used instead. This data set contains 217 points distributed over the whole range of growth temperatures.

DATA FOR GENERAL COMPARISON

In order to test the reliability of the models, 47 different data sets were collected (cf. Table 1) so as to cover a wide variety of micro-organisms including thermophilic strains (for example, *Thermus aquaticus* and *Bacillus stearothermophilus*), mesophilic strains (for example, *E. coli* and *Clostridium botulinum*) and psychrophilic strains (for example, *Vibrio psychroerythrus* and *Micrococcus cryophilus*). This data set contained prokaryotes and eukaryotes (for example. *Candida* sp. and *Gibberella fujikuroi*) grown on various defined and complex media. Data were obtained directly from tables where available, or deduced from the figures if their quality was satisfactory. To enable good parameter estimation, the number of points, for any one set, was never below 7.

Data Processing

HARDWARE AND SOFTWARE

All computations were done on a Macintosh IIfx computer with 8 Mo RAM and version 6·05 of the Mac/OS. Programmes were written in FORTRAN using

LANGUAGE SYSTEMS FORTRAN compiler 2.0 (Language Systems Corporation) under MPW 3.0 (Apple Computer Inc.). The graphical representations of model fits to data and of confidence regions were done with Statview II (Abacus Concepts Inc., Berkeley, CA) and SuperPaint 2.0 (Silicon Beach Software Inc., San Diego, CA).

PARAMETER ESTIMATION

The ordinary least square criterion was used to fit the model to the data. The sum of the squared residuals is noted as *SSR*. The smaller the *SSR*, the better the fit. The minimum *SSR* values (SSR_{min}) were computed in double precision with calls to IMSL 1.1 subroutine DUMINF (IMSL Inc., Houston, TX) which is a derivative-free modification (Brown & Dennis, 1972) of the usual Levenberg–Marquardt algorithm (Levenberg, 1944; Marquardt, 1963). Parameter starting values were chosen from a graphical examination of the data for the CTMI model. For the other models, when a parameter had no biological interpretation and no direct graphical counterpart, its starting value was chosen by an empirical trial and error approach. To check convergence with optimal parameter values, each computation was repeated with 16 different parameter starting values, located symmetrically on the corners of a hypercube centred on the first parameter estimate. The number of convergence failures during computation was used to appreciate the model's robustness as a routine tool.

PARAMETER CONFIDENCE LIMITS

Confidence regions ($\alpha = 0{\cdot}05$) for parameter values were defined according to Beale (1960) and determined with a previously described programme (Lobry *et al.*, 1991) in order that the parameter confidence limits should not be underestimated, as is the case with standard approximative marginal confidence limits.

PARAMETER VALUE ANALYSIS

A Principal Component Analysis on the CTMI model's four parameters was performed with STATVIEW II to analyse correlation between the parameters.

Results

PRELIMINARY COMPARISON OF THE FOUR MODELS ON BARBER'S DATA SET

Two kinds of difficulties during computation have been encountered with the RTK2 and ZWT models and with the H model especially. First, parameter starting values are difficult to set because of their lack of biological or graphical meaning. Second, the running of the fitting subroutine was not always satisfactory because of convergence failure or excessive execution time. These difficulties were partly

circumvented by trying new initial parameter values until success was achieved. On the other hand, these difficulties were never encountered in the CTMI model, since each parameter has a biological and graphical simple interpretation. Then, initial guesses for parameter values were easy to obtain.

The value of ordinary least square, SSR_{min}, is less for the CTMI model than the other three (Figs 1–4). However as SSR_{min} values are of the same order of magnitude, and since a single data set is involved, the four models can hardly be differentiated in this way. This point of view is corroborated by examination of model fits to data: no significant differences are visible from one graph to another.

Confidence regions show strong structural correlations between RTK2, ZWT and H model parameters. For the H model, a strong positive correlation is observed between A_1 and E_1, and negative correlations between E_1 and E_2, and A_1 and E_2. The RTK2 and the ZWT models show strong positive correlations between b and T_{min}, and negative correlations between c and b, c and T_{min}, and c and T_{max}. The high correlations are responsible for the difficulties encountered during computation. Moreover determination of parameter confidence limits is time-consuming, since they tend to become large (for example, parameters A_1 and E_1 in H model).

Unlike the previous models, the lack of structural correlation between parameters in the CTMI model allows for simple and accurate estimation of parameter values and their confidence limits: μ_{opt} spans 0·112 hr^{-1} (2·245–2·357); T_{min}: 4·5°C (2·5–7·0); T_{opt}: 0·72°C (40·90–41·62); and T_{max}: 0·55°C (47·25–47·80).

Hence, this preliminary comparison shows that the CTMI model is more convenient to use than the other three models: (i) biological interpretation of its parameters allows the setting of a simple parameter starting value; (ii) the lack of structural correlation between parameters enables rapid convergence to optimal parameter values and facilitates the determination of parameter confidence limits. However, the four models fit almost equally well to data. Excluding the H model, they were all considered in the following study. The H model was rejected because its parameters are all biologically meaningless, too time-consuming to estimate, and their confidence limits difficult to estimate accurately.

FULL COMPARISON ON 47 DIFFERENT DATA SETS

Difficulties during parameter estimation were encountered on nine occasions with the CTMI model, 13 occasions with the RTK2 model, and 24 occasions with the ZWT model. T_{min} and T_{max} are similar in all three models, but present some small differences. In general, the RTK2 model gives the smallest T_{min} estimates, followed by the ZWT model and then the CTMI. The RTK2 and ZWT models give larger T_{max} estimates than the CTMI model.

Table 2 shows, according to the ordinary least square criterion, that the CTMI model comes in first place 21 times and ten times in second place; that the RTK2 model comes in first place 20 times and seven times in second place; and that the ZWT model comes in first place six times and 30 times in second place. A Wilcoxon matched-pairs signed-rank test (Wilcoxon, 1945) shows that, at critical 5% level, the difference between the CTMI and ZWT models is significant ($P=0·02$), but not

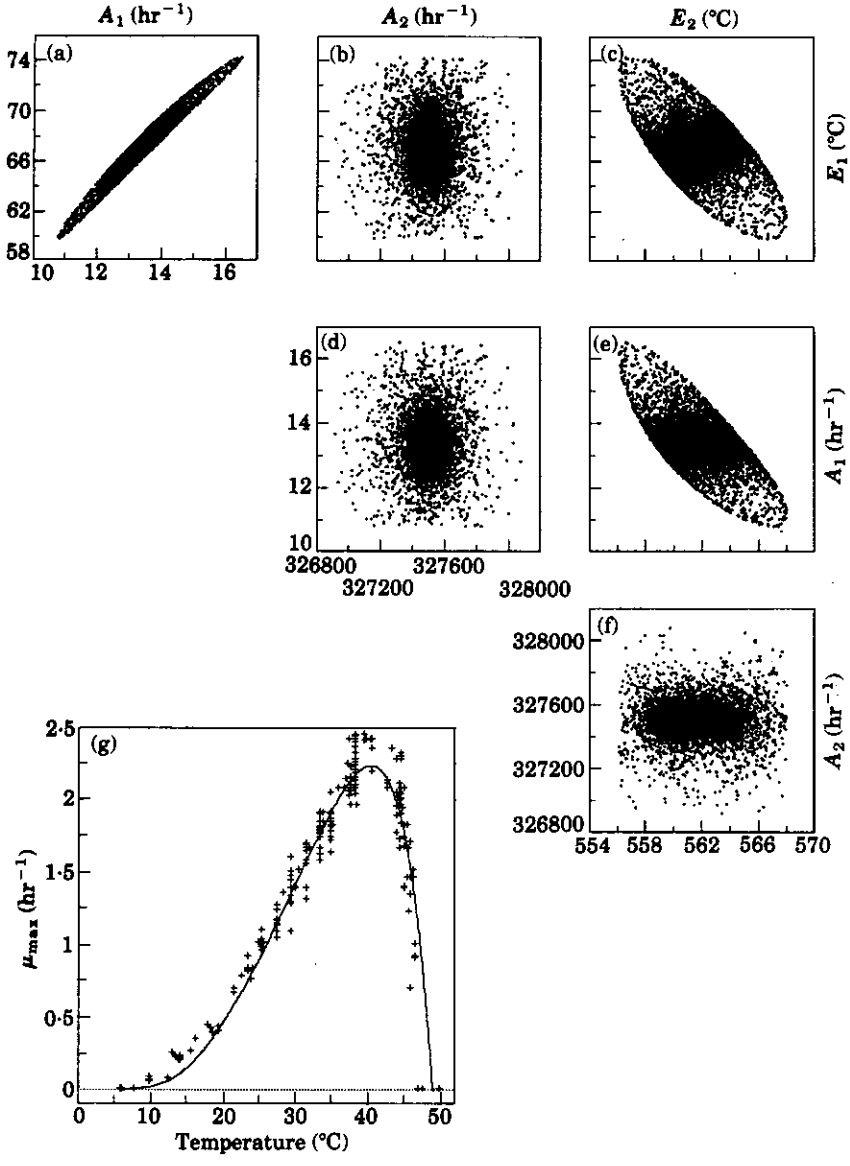

FIG. 1. H model. The best fit of the model to the Barber's data set is represented in (g). The confidence region for parameter values is projected on six different planes. Note the high structural correlation between parameters A_1 and E_1, E_2 and E_1, and E_2 and A_1. Parameter estimates and 95% limits are, respectively: $A_1 = 13{\cdot}29$ hr^{-1} (10·8–16·5); $E_1 = 66{\cdot}87$°C (59–74); $A_2 = 327509$ hr^{-1} (326950–328100); $E_2 =$ 561·87 °C (556–568). $SSR_{min} = 7{\cdot}442$.

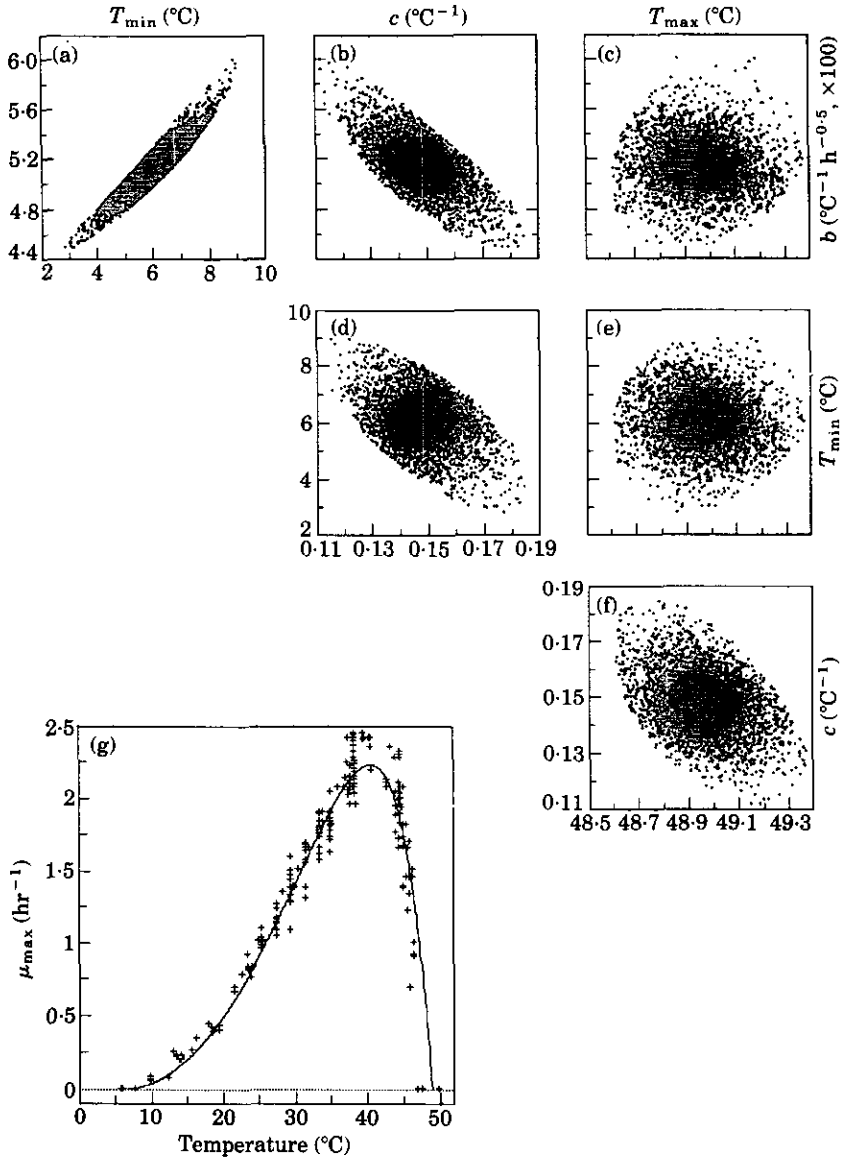

FIG. 2. ZWT model. The best fit of the model to the Barber's data set is represented in (g). The confidence region for parameter values is projected on six different planes. Note the high structural correlation between parameters T_{min} and b, c and b, and T_{min} and c. Parameter estimates and 95% limits are, respectively: $b = 0{\cdot}0514$°C^{-1} hr$^{-0.5}$ (0·045–0·060); $T_{min} = 6{\cdot}09$°C (2·5–9·0); $c = 0{\cdot}148$°C^{-1} (0·11–0·185); $T_{max} = 48{\cdot}95$°C (48·6–49·4). $SSR_{min} = 7{\cdot}459$.

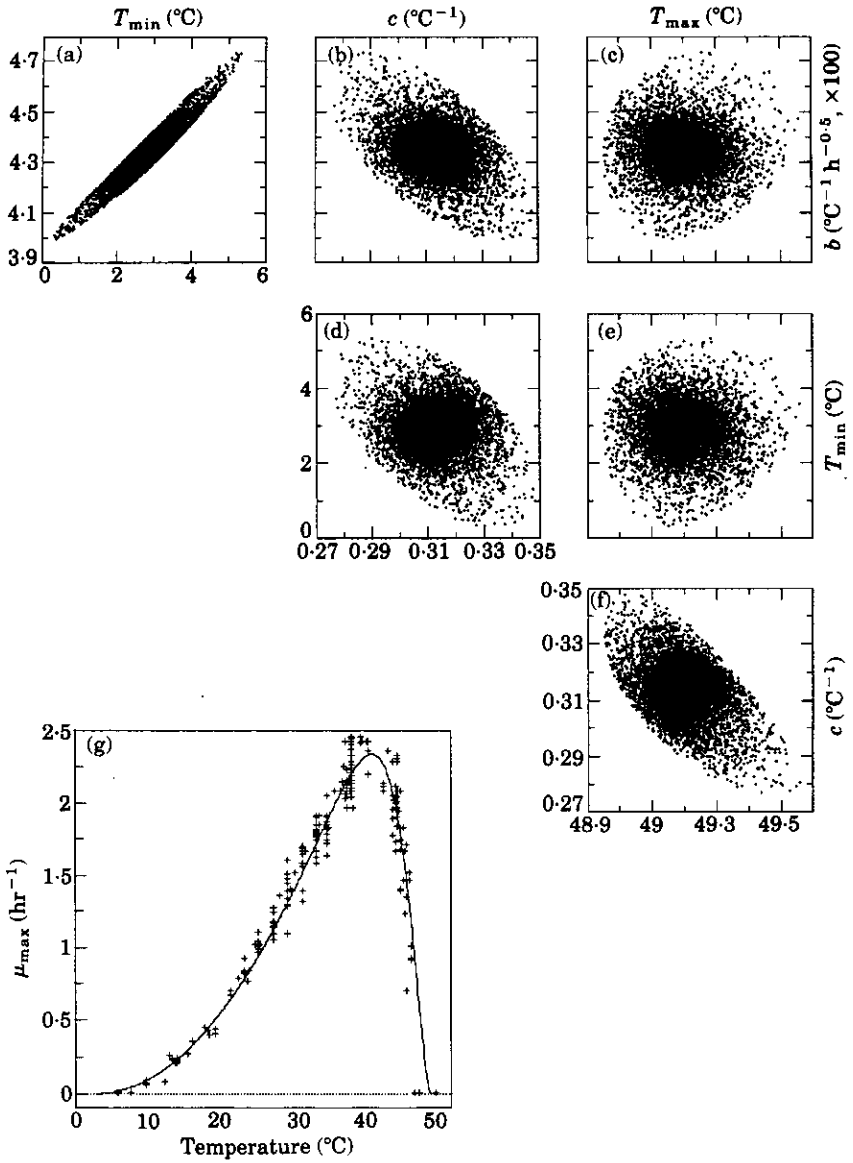

FIG. 3. RTK2 model. The best fit of the model to the Barber's data set is represented in (g). The confidence region for parameter values is projected on six different planes. Note the high structural correlation between parameters T_{min} and b. Parameter estimates and 95% limits are, respectively: b = 0·0435°C^{-1} hr$^{-0.5}$ (0·04–0·0475); T_{min} = 2·9°C (0·4–5·2); c = 0·314°C^{-1} (0·275–0·346); T_{max} = 49·2°C (48·96–49·5). SSR_{min} = 5·487.

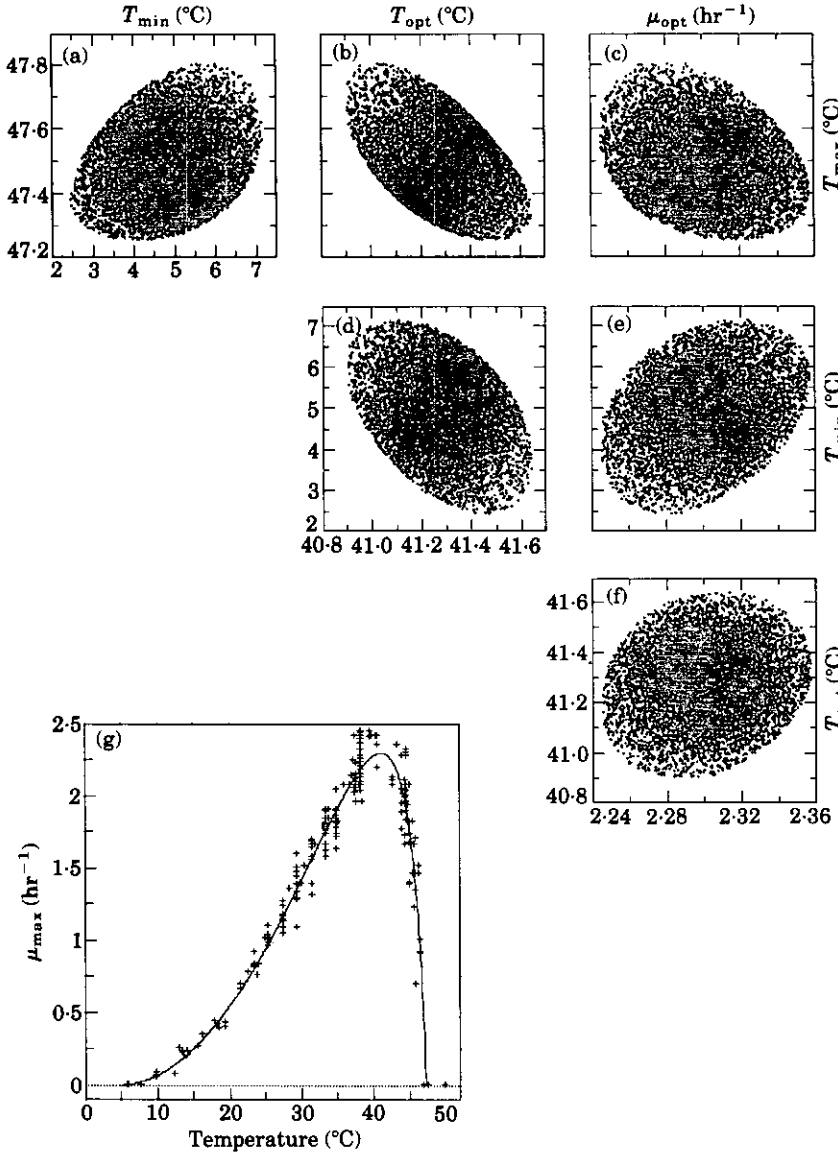

FIG. 4. CTMI model. The best fit of the model to the Barber's data set is represented in (g). The confidence region for parameter values is projected on six different planes. Note the absence of structural correlation between parameters. Parameter estimates and 95% limits are, respectively: $T_{min}=4{\cdot}9$°C (2·5–7); $T_{opt}=41{\cdot}3$°C (40·9–41·62); $T_{max}=47{\cdot}5$°C (47·25–47·8); $\mu_{opt}=2{\cdot}3$ hr^{-1} (2·245–2·357). $SSR_{min}=4{\cdot}747$.

TABLE 1

Description and sources of the data set used in computations

Code	Organism	Source	N.O.P.	T/G
Ab1	*Achromobacter sp.*483	Scott (1937)	8	T
Ab2	*Achromobacter sp.*5	Scott (1937)	7	T
Ab3	*Achromobacter sp.*7	Scott (1937)	8	T
Ac1	*Acinetobacter sp.*2.55	Ratkowsky *et al.* (1983)	17	G
Ac2	*Acinetobacter sp.*4.41	Ratkowsky *et al.* (1983)	26	G
Aer	*Aeromonas sp.*4.29	Ratkowsky *et al.* (1983)	19	G
Alt	*Alteromonas sp.* CLD38	Ratkowsky *et al.* (1983)	18	G
Bco	*Bacillus coagulans*	Ratkowsky *et al.* (1983)	18	G
Bme	*Bacillus megaterium*	Ratkowsky *et al.* (1983)	14	G
Bs1	*Bacillus stearothermophilus* 238	Ratkowsky *et al.* (1983)	14	G
Bs2	*Bacillus stearothermophilus*	Ratkowsky *et al.* (1983)	14	G
Bsu	*Bacillus subtilis*	Ratkowsky *et al.* (1983)	15	G
Cj1	*Cytophaga johnsonae*	Ratkowsky *et al.* (1983)	24	G
Cj2	*Cytophaga johnsonae*	Ratkowsky *et al.* (1983)	23	G
Can	*Candida* Y1	Scott (1937)	7	T
CbA	*Clostridium botulinum* type A	Ohye & Scott (1953)	9	T
CbB	*Clostridium botulinum* type B	Ohye & Scott (1953)	9	T
Ce1	*Cellulomonas sp.*	Baker (1974)	17	T
Ec1	*Escherichia coli*	Ratkowsky *et al.* (1983)	15	G
Ec2	*Escherichia coli* K-12	Ingraham (1958)	21	T
Ec3	*Escherichia coli*	Barber (1908)	217	T
Fla	*Flavobacterium sp.* 2.4	Ratkowsky *et al.* (1983)	21	G
Geo	*Geotrichoides sp.* Y9	Scott (1937)	7	T
Gf1	*Gibberella fujikuroi*	Borrow *et al.* (1964)	17	T
Gf2	*Gibberella fujikuroi*	Borrow *et al.* (1964)	17	T
Mc1	*Micrococcus cryophilus* ATCC15174	Tai & Jackson (1969)	10	T
Mc2	*Micrococcus cryophilus* M19	Tai & Jackson (1969)	15	T
Mc3	*Micrococcus cryophilus* T8	Tai & Jackson (1969)	14	T
Mor	*Moraxella sp.* 4.16	Ratkowsky *et al.* (1983)	24	G
Myc	*Mycotorula sp.* Y15	Scott (1937)	8	T
Pa1	*Pseudomonas aeruginosa*	Ratkowsky *et al.* (1983)	14	G
Pa2	*Pseudomonas aeruginosa* NRRL B23	Ingraham (1958)	11	T
Pf1	*Pseudomonas fluorescens*	Ratkowsky *et al.* (1983)	16	G
Pmo	*Pseudomonas morganii* M68	Ratkowsky *et al.* (1983)	18	G
Psy	*Pseudomonas syringae*	Young *et al.* (1977)	19	T
Pp1	*Pseudomonas psychrophiles* p-200	Ingraham (1958)	17	T
Pp2	*Pseudomonas psychrophiles* 1-3b	Ingraham (1958)	17	T
Pp3	*Pseudomonas psychrophiles* 31-3c	Ingraham (1958)	18	T
PI1	*Pseudomonas sp.* type I 4.54	Ratkowsky *et al.* (1983)	20	G
PI2	*Pseudomonas sp.* type I 6.4	Ratkowsky *et al.* (1983)	17	G
PII	*Pseudomonas sp.* type II 2.3	Ratkowsky *et al.* (1983)	17	G
Sma	*Serratia marcescens*	Ratkowsky *et al.* (1983)	10	G
Taq	*Thermus aquaticus* TY	Ratkowsky *et al.* (1983)	16	G
Vm1	*Vibrio marinus* ATCC15381	Ratkowsky *et al.* (1983)	8	G
Vm2	*Vibrio marinus* ATCC15382	Ratkowsky *et al.* (1983)	11	G
Vps	*Vibrio psychroerythrus* ATCC27364	Ratkowsky *et al.* (1983)	8	G
Xpr	*Xanthomonas pruni*	Young *et al.* (1977)	18	T

A code is attributed to each strain. NOP, number of points in the data set. T/G, tabulated or graphical material.

TABLE 2

Minimum of the sum of squared residuals obtained with the three models

Code	CTM1	RTK2	ZWT
Ab1	$6{\cdot}604 \cdot 10^{-4}$†	$9{\cdot}087 \cdot 10^{-4}$	$8{\cdot}644 \cdot 10^{-4}$
Ab2	$5{\cdot}134 \cdot 10^{-4}$†	$9{\cdot}645 \cdot 10^{-4}$	$7{\cdot}557 \cdot 10^{-4}$
Ab3	$3{\cdot}067 \cdot 10^{-4}$†	$4{\cdot}999 \cdot 10^{-4}$	$4{\cdot}350 \cdot 10^{-4}$
Ac1	$4{\cdot}260 \cdot 10^{-7}$	$2{\cdot}933 \cdot 10^{-7}$†	$3{\cdot}600 \cdot 10^{-7}$
Ac2	$2{\cdot}681 \cdot 10^{-7}$	$1{\cdot}767 \cdot 10^{-7}$	$1{\cdot}685 \cdot 10^{-7}$†
Aer	$9{\cdot}375 \cdot 10^{-7}$	$7{\cdot}822 \cdot 10^{-7}$†	$8{\cdot}627 \cdot 10^{-7}$
Alt	$1{\cdot}910 \cdot 10^{-5}$	$1{\cdot}658 \cdot 10^{-5}$†	$1{\cdot}720 \cdot 10^{-5}$
Bco	$1{\cdot}217 \cdot 10^{-1}$†	$1{\cdot}638 \cdot 10^{-1}$	$1{\cdot}387 \cdot 10^{-1}$
Bme	$3{\cdot}808 \cdot 10^{-2}$	$3{\cdot}816 \cdot 10^{-2}$	$3{\cdot}761 \cdot 10^{-2}$†
Bs1	$1{\cdot}375 \cdot 10^{-6}$†	$2{\cdot}909 \cdot 10^{-6}$	$1{\cdot}694 \cdot 10^{-6}$
Bs2	$2{\cdot}578 \cdot 10^{-1}$†	$3{\cdot}131 \cdot 10^{-1}$	$2{\cdot}873 \cdot 10^{-1}$
Bsu	$1{\cdot}998 \cdot 10^{-2}$†	$2{\cdot}509 \cdot 10^{-2}$	$1{\cdot}998 \cdot 10^{-2}$
Cj1	$4{\cdot}577 \cdot 10^{-4}$†	$5{\cdot}698 \cdot 10^{-4}$	$5{\cdot}189 \cdot 10^{-4}$
Cj2	$1{\cdot}832 \cdot 10^{-4}$†	$3{\cdot}387 \cdot 10^{-4}$	$2{\cdot}732 \cdot 10^{-4}$
Can	$1{\cdot}705 \cdot 10^{-4}$	$1{\cdot}344 \cdot 10^{-4}$†	$1{\cdot}399 \cdot 10^{-4}$
Cel	$5{\cdot}104 \cdot 10^{-3}$	$3{\cdot}974 \cdot 10^{-3}$†	$4{\cdot}151 \cdot 10^{-3}$
CbA	$1{\cdot}003 \cdot 10^{-3}$	$8{\cdot}429 \cdot 10^{-4}$	$5{\cdot}450 \cdot 10^{-4}$†
CbB	$9{\cdot}247 \cdot 10^{-3}$	$2{\cdot}789 \cdot 10^{-3}$†	$7{\cdot}392 \cdot 10^{-3}$
Ec1	$7{\cdot}127 \cdot 10^{-2}$†	$1{\cdot}294 \cdot 10^{-1}$	$9{\cdot}423 \cdot 10^{-2}$
Ec2	$9{\cdot}974 \cdot 10^{-2}$†	$1{\cdot}913 \cdot 10^{-1}$	$1{\cdot}130 \cdot 10^{-1}$
Ec3	4·747†	5·487	7·459
Fla	$6{\cdot}078 \cdot 10^{-8}$†	$1{\cdot}230 \cdot 10^{-7}$	$9{\cdot}433 \cdot 10^{-8}$
Geo	$1{\cdot}275 \cdot 10^{-4}$†	$2{\cdot}842 \cdot 10^{-4}$	$1{\cdot}677 \cdot 10^{-3}$
Gf1	$6{\cdot}675 \cdot 10^{-4}$	$9{\cdot}240 \cdot 10^{-4}$	$6{\cdot}318 \cdot 10^{-4}$†
Gf2	$4{\cdot}511 \cdot 10^{-4}$	$7{\cdot}124 \cdot 10^{-4}$	$3{\cdot}758 \cdot 10^{-4}$†
Mc1	$4{\cdot}470 \cdot 10^{-4}$†	$4{\cdot}896 \cdot 10^{-4}$	$5{\cdot}544 \cdot 10^{-4}$
Mc2	$6{\cdot}874 \cdot 10^{-3}$	$5{\cdot}063 \cdot 10^{-3}$†	$1{\cdot}392 \cdot 10^{-2}$
Mc3	$5{\cdot}901 \cdot 10^{-3}$	$4{\cdot}611 \cdot 10^{-3}$†	$9{\cdot}323 \cdot 10^{-3}$
Mor	$2{\cdot}750 \cdot 10^{-6}$	$1{\cdot}386 \cdot 10^{-6}$†	$2{\cdot}146 \cdot 10^{-6}$
Myc	$3{\cdot}682 \cdot 10^{-4}$	$3{\cdot}267 \cdot 10^{-4}$†	$3{\cdot}284 \cdot 10^{-4}$
Pa1	$3{\cdot}419 \cdot 10^{-2}$†	$3{\cdot}426 \cdot 10^{-2}$	$3{\cdot}479 \cdot 10^{-2}$
Pa2	$6{\cdot}751 \cdot 10^{-2}$†	$1{\cdot}243 \cdot 10^{-1}$	$7{\cdot}591 \cdot 10^{-2}$
Pf1	$4{\cdot}803 \cdot 10^{-2}$	$3{\cdot}457 \cdot 10^{-2}$†	$1{\cdot}008 \cdot 10^{-1}$
Pmo	$2{\cdot}726 \cdot 10^{-7}$	$3{\cdot}068 \cdot 10^{-7}$	$2{\cdot}717 \cdot 10^{-7}$†
Psy	$1{\cdot}126 \cdot 10^{-2}$	$5{\cdot}295 \cdot 10^{-3}$†	$8{\cdot}979 \cdot 10^{-3}$
Pp1	$1{\cdot}713 \cdot 10^{-2}$	$1{\cdot}514 \cdot 10^{-2}$†	$1{\cdot}792 \cdot 10^{-2}$
Pp2	$1{\cdot}696 \cdot 10^{-2}$†	$1{\cdot}993 \cdot 10^{-2}$	$1{\cdot}869 \cdot 10^{-2}$
Pp3	$1{\cdot}526 \cdot 10^{-2}$	$1{\cdot}096 \cdot 10^{-2}$†	$2{\cdot}600 \cdot 10^{-2}$
PI1	$2{\cdot}667 \cdot 10^{-7}$	$2{\cdot}297 \cdot 10^{-7}$†	$2{\cdot}370 \cdot 10^{-7}$
PI2	$1{\cdot}204 \cdot 10^{-7}$†	$2{\cdot}426 \cdot 10^{-7}$	$2{\cdot}124 \cdot 10^{-7}$
PII	$5{\cdot}974 \cdot 10^{-8}$	$4{\cdot}672 \cdot 10^{-8}$†	$5{\cdot}260 \cdot 10^{-8}$
Sma	$1{\cdot}919 \cdot 10^{-2}$†	$3{\cdot}370 \cdot 10^{-2}$	$2{\cdot}653 \cdot 10^{-2}$
Taq	$9{\cdot}268 \cdot 10^{-3}$	$8{\cdot}275 \cdot 10^{-3}$†	$8{\cdot}898 \cdot 10^{-3}$
Vm1	$4{\cdot}731 \cdot 10^{-4}$†	$5{\cdot}518 \cdot 10^{-4}$	$1{\cdot}377 \cdot 10^{-3}$
Vm2	$3{\cdot}906 \cdot 10^{-3}$	$3{\cdot}675 \cdot 10^{-3}$†	$3{\cdot}756 \cdot 10^{-3}$
Vps	$9{\cdot}035 \cdot 10^{-5}$	$6{\cdot}713 \cdot 10^{-5}$†	$4{\cdot}108 \cdot 10^{-4}$
Xpr	$1{\cdot}126 \cdot 10^{-2}$	$7{\cdot}871 \cdot 10^{-3}$†	$9{\cdot}343 \cdot 10^{-3}$

† Indicates the smallest value.

between the CTMI and RTK2 models ($P=0{\cdot}33$) or between the RTK2 and ZWT models ($P=0{\cdot}72$). Hence, according to the ordinary least-square criterion, only the ZWT model appears to be inadequate.

The ZWT model was rejected because of its relatively bad fit to data as compared to the CTMI model and, for the sake of convenience, to avoid difficulties during parameter estimation. The RTK2 was rejected for reasons of convenience only: two of its parameters are biologically meaningless and this provides no help in results analysis.

BIOLOGICAL ANALYSIS OF CTMI RESULTS

The three cardinal temperatures and the maximum specific growth rate at the optimal temperature estimates are summarized in Table 3. In general, the precision of the estimate is about ± 5°C for T_{min}, ± 2°C for T_{opt}, ± 1°C for T_{max}, and ± 10% for μ_{opt}.

Principal components analysis of these data shows that 95·5% of variance can be summarized by two factors. The first is a linear combination of the three cardinal temperatures, whereas the second is more linked to μ_{opt}. The three cardinal temperatures are then highly correlated, and μ_{opt} seems to be independent.

This high linear correlation between cardinal temperatures was unexpected and, consequently, the relationships between them were considered individually (Fig. 5). The high linear correlation between the cardinal temperatures observed was summarized by linear orthogonal regression, since both variables are subject to variance. The linear relationship is especially close between T_{max} and T_{opt} ($r=0{\cdot}991$). All the strains present in the data set seem to follow these relationships, except for the three strains of *Vibrio* sp. for which T_{min} is greater than expected.

Discussion and Conclusion

MECHANISTIC APPROACH FAILURE

The Hinshelwood model was elaborated from a mechanistic approach to microbial growth based on a single growth rate-determining reaction. As pointed out by Heitzer *et al.* (1991), the problems associated with interpreting the Hinshelwood model parameters are similar to those encountered in Monod's growth kinetics (Monod, 1941). Monod's growth kinetics use K_s values that are mathematically analogous to the Michaelis–Menten K_m values (Michaelis & Menten, 1913). The analogy between the Monod model and the Michaelis–Menten model has to be made with caution because the homologous parameter does not have the same significance (Lobry *et al.*, 1992). The same applies for the parameters in the Hinshelwood model which are analogous to the thermodynamic constants of the Arrhénius chemical model (Arrhénius, 1889). However, these parameters can no longer be interpreted as true thermodynamic properties unless a single growth rate-determining reaction can be identified. This hypothesis has been frequently criticized (Burton, 1936; Teissier, 1936; Monod, 1941; Senez, 1962; Marr, 1991). Moreover, Hinshelwood's model is

TABLE 3

Estimation of the CTMI model parameters, with 95% confidence limits within brackets

Code	T_{min} (°C)	T_{opt} (°C)	T_{max} (°C)	μ_{opt} (hr^{-1})
Ab1	−10·0 [−16;−5·0]	26·2 [24·4;28·5]	31·5 [30·1;35·0]	6·6 10^{-1} [5·9;7·7]
Ab2	−7·8 [−16;−2·0]	24·6 [22·4;29·5]	30·0 [27·5;30·3]	5·8 10^{-1} [5·2;7·3]
Ab3	−10·5 [−15;−7·0]	25·3 [24·0;26·8]	32·4 [30·7;35·3]	5·9 10^{-1} [5·6;6·5]
Ac1	1·4 [−3·0;4·9]	28·4 [27·7;29·2]	35·1 [34·6;35·8]	6·3 10^{-3} [6·1;6·6]
Ac2	0·1 [−1·0;1·1]	30·2 [29·9;30·6]	37·3 [37·0;37·6]	8·3 10^{-3} [8·2;8·5]
Aer	5·1 [1·5;8·0]	35·4 [34·5;36·4]	44·0 [42·5;46·5]	8·7 10^{-3} [8·3;9·2]
Alt	−5·2 [−7·6;−3·5]	25·5 [25·0;26·1]	33·4 [32·0;35·5]	7·5 10^{-2} [7·3;7·8]
Bco	12·1 [−13;30]	53·8 [48·5;57·5]	62·9 [61·0;71·0]	1·0 [0·9;1·2]
Bme	19·8 [12;26]	50·3 [49·0;51·6]	59·2 [57·0;65·0]	1·6 [1·5;1·8]
Bs1	30·8 [29;33]	57·2 [56·5;57·9]	65·5 [65·1;66·0]	1·8 10^{-2} [1·7;1·8]
Bs2	33·6 [23;42]	64·8 [62·5;67·5]	71·8 [70·0;81·0]	3·0 [2·6;3·3]
Bsu	13·4 [8·0;17]	38·7 [37·5;40·0]	51·0 [50·3;52·2]	1·2 [1·1;1·2]
Cj1	−11·3 [−24;−2·5]	24·9 [22·4;26·9]	32·0 [30·9;34·6]	5·4 10^{-2} [4·8;5·9]
Cj2	−7·5 [−13;−3·0]	27·8 [26·5;29·0]	33·1 [32·8;33·8]	6·2 10^{-2} [5·8;6·7]
Can	−9·7 [−20;−2·0]	21·8 [19·0;24·3]	26·8 [25·0;44·0]	2·5 10^{-1} [2·0;3·1]
Cel	−2·9 [−11;2·6]	25·4 [23·9;27·3]	29·0 [28·5;30·8]	3·0 10^{-1} [2·7;3·4]
CbA	11·0 [8·8;13]	39·3 [38·7;40·0]	45·8 [45·6;46·2]	1·1 [1·0;1·1]
CbB	13·0 [7·0;17]	37·9 [36·0;39·8]	46·1 [45·3;47·8]	1·0 [0·9;1·2]
Ec1	5·6 [−7·5;13]	40·3 [38·4;41·8]	47·3 [46·7;48·5]	1·4 [1·3;1·6]
Ec2	11·2 [7·4;14]	41·0 [40·1;42·0]	48·0 [47·0;48·4]	2·1 [1·9;2·2]
Ec3	4·9 [2·5;7·0]	41·3 [40·9;41·6]	47·5 [47·3;47·8]	2·3 [2·2;2·4]
Fla	−2·8 [−4·7;−1·0]	29·3 [28·9;29·8]	35·5 [35·1;36·0]	4·3 10^{-3} [4·2;4·4]
Geo	−9·0 [−16;−2·4]	20·1 [17·5;21·2]	26·4 [25·8;30·0]	2·4 10^{-1} [2·0;2·7]
Gf1	2·3 [−0·9;5·0]	30·8 [30·0;31·7]	39·7 [39·0;40·4]	2·4 10^{-1} [2·3;2·6]
Gf2	1·5 [−1·0;3·9]	30·9 [30·2;31·6]	39·8 [39·2;40·3]	2·4 10^{-1} [2·3;2·5]
Mc1	−7·1 [−12;−2·2]	21·5 [20·0;22·3]	25·5 [25·2;27·3]	2·9 10^{-1} [2·6;3·2]
Mc2	0·9 [−8·0;6·7]	30·9 [28·8;32·8]	37·9 [37·2;40·0]	3·8 10^{-1} [3·3;4·3]
Mc3	0·1 [−10;6·0]	28·3 [26·4;30·2]	35·2 [34·3;37·8]	3·4 10^{-1} [2·8;3·9]
Mor	−0·9 [−8·0;6·5]	30·5 [22·3;32·0]	37·2 [36·6;40·1]	6·3 10^{-3} [5·5;6·8]
Myc	−5·7 [−11;1·9]	28·4 [26·5;30·5]	30·7 [30·0;44·0]	4·3 10^{-1} [3·6;5·2]
Pa1	5·0 [−13;13]	38·1 [36·5;40·7]	46·2 [45·0;47·1]	1·1 [1·0;1·2]
Pa2	4·4 [−7·0;14]	40·3 [38·3;42·5]	46·0 [45·2;46·6]	1·7 [1·5;2·0]
Pf1	9·8 [3·0;15]	38·6 [37·3;39·9]	45·4 [44·8;46·2]	1·2 [1·1;1·3]
Pmo	2·1 [−2·0;5·0]	36·7 [36·1;37·3]	42·6 [42·2;43·1]	6·4 10^{-3} [6·1;6·6]
Psy	−1·8 [−9·0;3·5]	27·8 [26·5;29·5]	36·3 [35·0;37·1]	5·1 10^{-1} [4·7;5·7]
Pp1	−5·0 [−9·5;−1·0]	31·6 [30·4;32·4]	35·1 [34·9;36·2]	8·9 10^{-1} [8·2;9·7]
Pp2	−10·1 [−17;−5·0]	30·8 [29·3;33·3]	34·0 [33·2;34·2]	8·0 10^{-1} [7·3;9·1]
Pp3	−5·0 [−9·3;−1·0]	29·8 [28·2;30·8]	34·3 [34·1;34·7]	9·6 10^{-1} [8·4;10]
Pl1	−0·5 [−4·0;2·5]	30·0 [29·3;30·8]	34·4 [33·6;36·0]	4·3 10^{-3} [4·0;4·6]
Pl2	−3·5 [−7·0;−0·4]	26·0 [25·4;26·6]	29·4 [29·1;30·2]	4·7 10^{-3} [4·6;4·9]
Pll	−2·9 [−7·0;0·5]	27·2 [26·6;27·8]	34·4 [33·5;35·8]	3·7 10^{-3} [3·6;3·8]
Sma	−2·1 [−15;5·5]	25·8 [24·1;27·7]	31·3 [30·9;32·5]	1·3 [1·2;1·5]
Taq	36·8 [26;45]	70·9 [68·7;73·0]	81·8 [79·4;89·1]	4·5 10^{-1} [4·1;5·1]
Vm1	2·7 [−7·5;7·3]	15·6 [13·9;16·8]	19·5 [19·1;20·9]	2·1 10^{-1} [1·9;2·5]
Vm2	6·5 [2·8;9·4]	23·8 [22·9;24·8]	29·7 [28·9;30·5]	5·1 10^{-1} [4·7;5·5]
Vps	3·8 [−2·0;6·0]	12·4 [11·6;13·8]	18·5 [17·5;19·6]	1·1 10^{-1} [0·9;1·2]
Xpr	1·1 [−6·5;6·5]	30·8 [29·3;32·2]	36·8 [36·2;38·3]	4·4 10^{-1} [3·9;4·9]

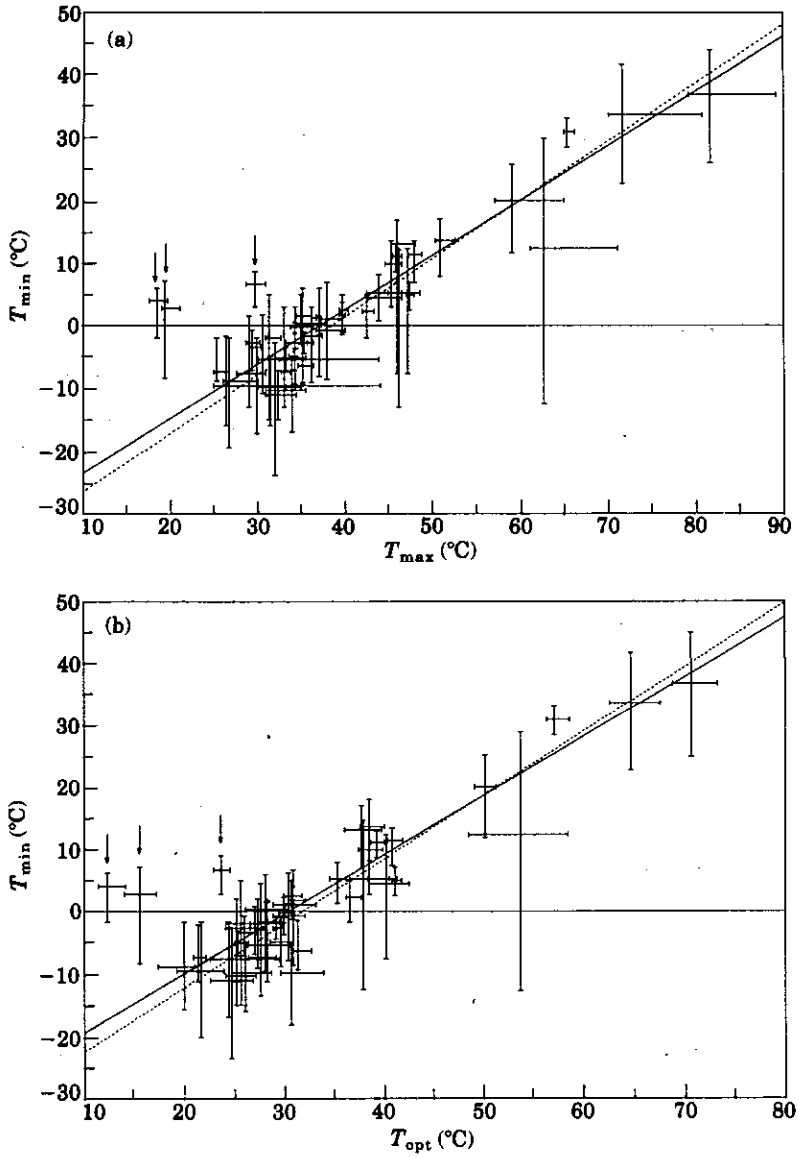

FIG. 5. Representation of the cardinal temperatures for 47 different strains according to the CTMI model. The length of the crosses represents the confidence limits for parameter values ($\alpha = 0{\cdot}05$). The lines represent the linear orthogonal regression for all stains. The broken lines represent the linear orthogonal regression when the three *Vibrio* sp. (indicated by an arrow) are removed. (a) $T_{min} = 0{\cdot}866 T_{max} - 31{\cdot}687$, $r = 0{\cdot}871$; (b) $T_{min} = 0{\cdot}953 T_{opt} - 28{\cdot}913$, $r = 0{\cdot}859$; (c) $T_{max} = 1{\cdot}101 T_{opt} + 3{\cdot}203$, $r = 0{\cdot}991$.

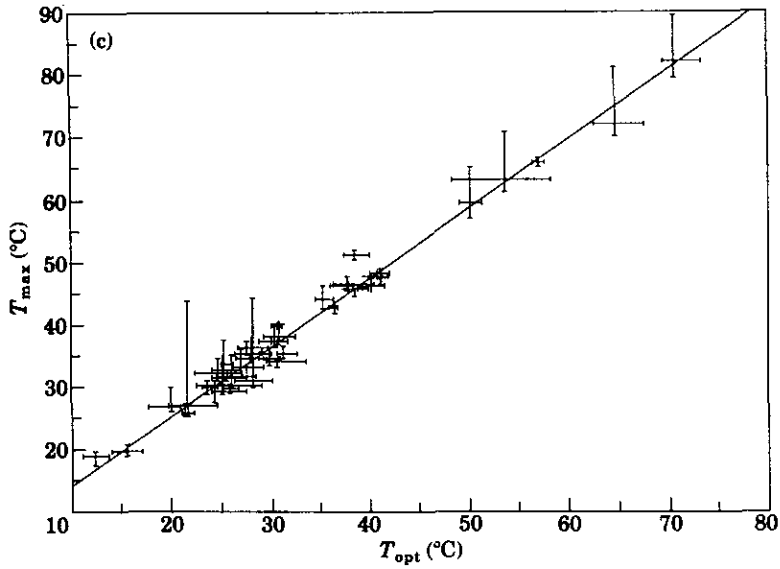

FIG. 5—continued.

the least adapted to the data (a fact also reported by Ratkowsky *et al.*, 1982). Hence, here, the mechanistic approach appears to be unsuitable and error-prone.

DESCRIPTIVE APPROACH INTEREST

The CTMI model is a purely descriptive model: instead of the other models it uses only simple biologically meaningful constants as parameters. Microbiologists are familiar with the concept of cardinal temperatures T_{min}, T_{opt} and T_{max}; the parameter μ_{opt} is the maximum specific growth rate at the optimal temperature. For this reason the CTMI model is easy to use by biologists. Moreover, all parameters have a graphical interpretation so that they can be read directly when plotting μ_{max} versus temperature. Last, the lack of structural correlation between parameters in the CTMI model is an interesting property: structural correlation between parameters should be avoided as it gives large confidence limits for parameter values, making parameter estimations and comparisons difficult. Hence, here, the descriptive approach has provided a useful model.

LINEAR CORRELATION BETWEEN CARDINAL TEMPERATURES

There is a pronounced linear correlation observed between cardinal temperatures in 47 strains. As the temperature permissive range of these strains spans 80°C and the growth culture conditions are very different, it might be expected that the observed relationships would be general. However, a more extensive analysis is required to confirm these results and detect those strains (such as the halophilic *Vibrio* analysed

here) with atypical growth temperature responses. As far as we know, this is the first time that this linear correlation between cardinal temperatures has been described, although the fact that the optimal temperature is usually only a few degrees off the maximum temperature is well known. This new relationship was obtained thanks to the CTMI model because it allows the estimations of the three cardinal temperatures with their confidence limits.

According to the linear correlations between the cardinal temperatures, one temperature is enough to describe the growth permissive temperature range instead of the three cardinal temperatures. This would be in the spirit of Occam's razor which states that entities are not to be multiplied beyond necessity. This would also reduce the experimental effort when characterizing the effect of temperature on the growth of a microbial strain. This simplification could, however, be dangerous as there would be a loss of information if the proportion of atypical strains (for example, *Vibrio*) is large. But in some works indeed, only one cardinal temperature is used for taxonomic purposes (Senez, 1968). Lastly, the reason and significance of this puzzling linear correlation between cardinal temperatures remain to be investigated.

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