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Development of a predictive model to describe the effects of temperature and water activity on the growth of spoilage pseudomonads

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Abstract

A combined temperature and water activity model for the growth of psychrotrophic pseudomonads was developed using turbidimetic data. Psychrotrophic pseudomonads were isolated from various modified and whole milks. The fastest growing strain was identified and used to develop the model. Generation time estimates calculated by turbidimetric and viable count data differed but this difference was constant with respect to temperature and was incorporated into the modelling process so that all models are constructed to predict generation times equivalent to those calculated by viable counts, the standard method for enumerating microorganisms in food products. © 1997 Elsevier Science B.V.

Keywords: Psychrotrophic; Pseudomonad; Predictive microbiology; Temperature; Water activity

1. Introduction

Psychrotrophic pseudomonads are the major spoilage organisms of aerobic chill-stored food products with neutral pH and high water activity, such as milk, cream and fresh meat (Chandler and McMeekin, 1985; Shelley et al., 1987; Tatini et al., 1991). By developing a model for the growth rate of psychrotrophic pseudomonads, the rate of spoilage of various products can, in theory, be predicted by monitoring the product temperature and water activity. Benefits of such predictive models in food microbiology are numerous and include predicting shelf life, assessing the hygienic efficiency of processing and distribution, determining the effect of lapses in storage conditions and determining the microbiological safety of a product (McMeekin et al., 1993).

The amount of data required to generate reliable models have led some researchers to use less time-consuming, and often indirect, methods of data collection, such as turbidimetry in laboratory media rather than traditional (viable count) methods. The concern, that the use of indirect methods for growth curve generation may result in generation times different to that determined using viable counts, was suggested in Baranyi et al. (1993) and Dalgaard et al.

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(1994). This is not a new observation. Monod (1949) commented that although spectrophotometers were becoming widely used since their introduction in 1935, 'not enough efforts have been made to check them against direct estimations of cell concentrations or bacterial densities'.

Inconsistencies between models for the same bacterium may be a result of the primary model used to determine generation time. The observation by several workers (Whiting and Cygnarowicz-Provost, 1992; Baranyi et al., 1993; Ross, 1993; Dalgaard et al., 1994), that generation times calculated from the Gompertz function and 'traditional' steepest tangent methods disagree is an example. Baranyi et al. (1993) attributed this to the modified-Gompertz function having a definite curvature around its point of inflection, resulting in a higher growth rate than expected. Ross (1993), in a simulation study, found the modified-Gompertz equation to underpredict the generation time by an average of 13% and therefore, proposed a correction factor to relate generation times estimated by the Gompertz function to those by other methods.

In the present study, a secondary model describing the growth of psychrotrophic pseudomonads in response to water activity and temperature was developed using Bělehrádek-type models, such as those proposed by Ratkowsky et al. (1982, 1983) and McMeekin et al. (1987). Growth rates, determined using turbidimetric methods, were correlated to those obtained using viable count methods. Validation of the psychrotrophic pseudomonad model is described in Neumeyer et al. (1997).

2. Materials and methods

2.1. Strain isolation

CSIRO Dairy Research Laboratories in Victoria, Australia, provided nine strains of psychrotrophic pseudomonads of dairy origin. Other pseudomonads were isolated from pasteurised modified milk by allowing cartons, purchased at retail outlets, to spoil at 4 and 10°C. One ml samples were removed at spoilage, serially diluted in 0.1% peptone (Oxoid L37), plated onto *Pseudomonas* selective agar (Oxoid CM559) and incubated at 25°C for 2 days. Presumptive pseudomonad isolates were confirmed using Analytical Profile Index (API) 20NE strips (Biomérieux sa, Lyon, France). Isolates were maintained both in short term storage for 1–2 months (using slopes of PCA) and long term storage (on beads in 15% glycerol (v/v) glycerol in nutrient broth No. 2 at -70°C).

2.2. Preparation of inocula

Inocula were grown at 25°C for 2 days in 30 ml nutrient broth No. 2 (Oxoid CM67) in a 150 ml flask. These conditions ensure cultures to be in the stationary phase at the completion of incubation (Neumeyer, 1995).

2.3. Preparation of media

For temperature studies, nutrient broth No. 2 ($a_w = 0.996$) was prepared according to the manufacturer's instructions. For water activity studies, 500 ml of nutrient broth No. 2 (Broth A; 0.66% NaCl (w/w)) was prepared. A second batch of nutrient broth No. 2 containing 40 g NaCl was prepared (Broth B; 8.40% NaCl (w/w)). Broths A and B were then mixed aseptically in sterile, capped, L-shaped, glass culture vessels (L-tubes), in various ratios to achieve the range of water activities required (i.e. from 0.996 to 0.947). The water activity of each broth was calculated from the tables of Resnik and Chirife (1988) and using the formula of Robinson and Stokes (1949). The water activity of each broth was also measured using a dew-point water activity meter (Aqualab CX2, Decagon Devices, Pullman, WA). The growth rate at each water activity was determined in duplicate and the generation times calculated from duplicate tubes averaged.

2.4. Temperature gradient incubator protocols

L-tubes containing 10 ml nutrient broth No. 2 were placed in a temperature gradient incubator (TGI; Model TN3, Toyo Roshi, USA) $\approx$12 h prior to inoculation to allow temperature equilibration. Sufficient inoculum was added to each L-tube to reduce the %Transmittance (%T) to $\approx$80% (usually 200–300 μl). The amount of inoculum added was constant for each experiment. The time of each inoculation was recorded. Temperature fluctuations within each L-tube were reduced by operating the

TGI in a constant temperature room and were typically ±0.5°C of the reported value. L-tubes were agitated (≈40 oscillations per min) to minimise the formation of oxygen gradients. At intervals after inoculation, %T was measured using a spectrophotometer (Spectronic 20, Milton Roy, USA) at 540 nm. Growth curves were considered complete when the %T had dropped to 5–7%T. A minimum of 15 readings were recorded per growth curve. At the cessation of growth, the temperature in each tube was recorded five times using an electronic Fluke thermometer (Model 51K/J, John Fluke, IL).

2.5. Calculation of generation times

The Gompertz function was applied to both log(CFU) data and %T data to enable objective determination of growth rates. The function and interpretation of its parameters are:

$$Y_t = A + D\exp\{-\exp[-B(t-M)]\} \quad (1)$$

in which A = value of the lower asymptote (i.e. $Y_{(-\infty)}$); D = difference in value of the upper and lower asymptote (i.e. $Y_{(\infty)} - Y_{(-\infty)}$); M = time at which the absolute growth rate is maximal; BD/e = slope of the tangent to the curve at M in the case of viable count data; Y_t = population density in log(CFU) at time, t and in the case of %T data; Y_t = %T of the culture at time, t. The various phases of growth such as length of lag phase, generation time, exponential growth rate and maximum population density are calculated from the parameters A, B, D and M. The equation for generation time (viable count data), incorporating the correction factor of Ross (1993) is

$$\text{generation time} = \frac{e\log 2 \times 1.13}{BD} = 0.925/BD \quad (2a)$$

where BD/e is the steepest slope of the curve, which occurs at time = (M. McMeekin et al., 1993; Appendix 2A.9) showed that within the range 20 to 60%T the relationship between %T and log CFU/ml is linear and a doubling of the population is represented by a 24.5% decrease in %T. By plotting change in %T versus time, a sigmoid curve results from which an estimate of the generation times can be obtained.

$$\begin{aligned}\text{generation time } (\%T) &= (24.5 * e * 1.13)/BD \\ &= (24.5 * 2.71828 * 1.13)/BD \\ &= 72.26/BD \end{aligned} \quad (2b)$$

where B and D are the Gompertz parameters estimated by fitting the function (Eq. (1)) to %T data.

2.6. Strain selection

Those strains which grow the fastest at refrigeration temperatures, will be the most significant in the spoilage association of milk and dairy products. To estimate the strain to strain variability of growth rate, and to select the strains for modelling studies, the growth rates of the psychrotrophic pseudomonad isolates were determined, in quadruplicate, by growing them in nutrient broth No. 2 at 10.8°C. Initial selection was at ≈10°C, as this is an abuse temperature often encountered by milk and milk-products. Growth was monitored using turbidimetric methods and generation times calculated using the protocols described above.

2.7. Data generation

2.7.1. Temperature models

Pseudomonas putida 1442 was the primary strain used for modelling purposes. A number of other strains (*P. fluorescens* 1412, *P. fragi* NCIMB 8542, *P. fragi* I6 and a cocktail of five strains consisting of *P. putida* 1442, *P. fluorescens* 1412, *P. fragi* I6, *P. fluorescens* I1 and *P. fluorescens* I8.2) were also studied in nutrient broth No. 2 at constant water activity and at ≈1°C intervals (for 0–30°C temperature range) or 0.5°C intervals (for 0–15°C temperature range). Generation times were calculated from Eq. (2a) for VC data and Eq. (2b) for %T data. Data were fitted by linear regression to the Bělehrádek-type model of Ratkowsky et al. (1982):

$$\sqrt{r} = b(T - T_{\min}) \quad (3)$$

where r = rate at T; T = temperature; $T_{\min}$ = a theoretical minimum temperature for growth at which growth rate is predicted to be zero; b = the slope of the regression line.

The data for *P. putida* 1442 was also fitted using non-linear regression. *P. putida* 1442 was also monitored over the temperature range 20–40°C to provide

additional growth response data, so that the biokinetic range could be modelled using the extended Bělehrádek-type model of Ratkowsky et al. (1983) and fitted using non-linear regression:

$$\sqrt{r} = b'(T - T_{min})\{1 - \exp[c(T - T_{max})]\} \quad (4)$$

where T_{max} = a theoretical maximum temperature for growth, analogous to T_{min}, at which growth rate is predicted to be zero; b' = a coefficient related to the slope of the regression line; c = coefficient to be estimated and r, T and T_{min} as for Eq. (3). All non-linear regressions were fitted using PROC NLIN of SAS (1989).

2.7.2. Water activity models

Growth rate data were generated for *P. putida* 1442, *P. fluorescens* 1412, *P. fragi* NCIMB 8542, *P. putida* 1261 and *P. fluorescens* 8.2 as a function of water activity. Generation times were calculated from parameters derived from Eqs. (2a) and (2b), while regression equations were fitted by non-linear regression to the Bělehrádek-type model of McMeekin et al. (1987):

$$\sqrt{r} = b''\sqrt{(a_w - a_{w_{min}})} \quad (5)$$

where b'' = slope of the regression line; a_w = water activity; $a_{w_{min}}$ = theoretical minimum a_w for growth at which growth rate is predicted to be zero.

2.8. Model calibration

2.8.1. Correlation of viable count and turbidimetric methods

P. putida 1442 inoculum was prepared as in Section 2.2. L-tubes containing 15 ml nutrient broth No. 2 were inoculated with $\approx 10^5$ cfu/ml. During growth, both %T and VC were monitored simultaneously for each culture, as described in Section 2.4 and Section 2.8.2. Generation times were calculated as in 2.5 for %T ($GT_{\%T}$) and VC (GT_{VC}) data, respectively.

2.8.2. Viable count measurements

At each sample time, 0.1 ml aliquots of the culture were removed and serially diluted in 0.1% peptone. 0.1 ml aliquots of appropriate dilutions were plated in duplicate onto Plate Count Agar (Oxoid CM463). After incubation at 25°C for 2 days, plates with between 30 and 300 colony forming units (cfu) were counted. Log cfu/ml values were calculated from these data using the method described by Farmiloe et al. (1954) i.e.

$$\text{CFU/ml or gram} = \frac{C}{V(n_1 + 0.1n_2)f}$$

where C = total number of colonies counted; V = volume of inoculum applied to each plate; n_1 = number of plates counted at the lower dilution; n_2 = number of plates counted at the higher dilution; f = dilution factor for the higher dilution. A total of 10–15 samples were taken throughout each growth curve. Fewer readings were taken, compared to the turbidimetric method, due to workload constraints.

3. Results

3.1. Strain selection

$GT_{\%T}$ at 10.8°C ranged from 3.3 to 5.1 h with an average GT (±SD) of 4.2±0.5 h. The two fastest strains were *P. putida* 1442 and *P. fragi* NCIMB 8542, both with a generation time of 3.3 h. *P. putida* 1442 was selected as the primary isolate for modelling purposes as the origin of *P. fragi* NCIMB 8542 was unknown (S. Colombo, ATCC, USA personal communication). *P. fluorescens* 1412, as one of the slowest strains, was also modelled to provide an indication of the variability of growth rates of psychrotrophic pseudomonad isolates.

3.2. Temperature models

Fig. 1 describes the growth rate data for *P. putida* 1442 as a square root plot and combines data from two experiments, one in the temperature range 0–15°C and the other 0–30°C. This is a good example of the high degree of repeatability possible when using turbidimetric methods. The response of *P. putida* 1442 is representative of the other strains studied. Fitted model parameters are summarised in Table 1. For each isolate between 26–30 growth curves were monitored over the temperature range 0–30°C. High r^2 values were noted, the lowest being the cocktail of five strains with an r^2 value of 0.990.

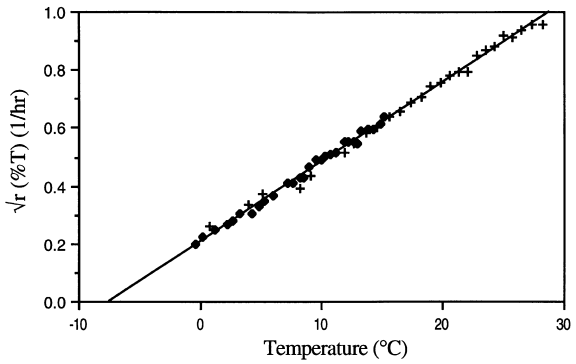

Fig. 1. Square root plot of *P. putida* 1442. $\sqrt{r_{\%T}} = 0.027529$ (T + 7.6), $r^2 = 0.996$ using two datasets; one (♦) at temperatures 0–15°C ($n = 29$) and the other (+) at 0–30°C ($n = 26$).

The model for *P. putida* 1442 over the entire biokinetic range (Eq. (4)) and described in terms of $\%T$ is

$$\sqrt{r_{\%T}} = 0.0309(T + 6.0)$$
$$\{1 - \exp[0.1717(T - 41.2]$$
$$n = 85 \qquad \text{RMSE} = 0.0331$$

where n = the number of growth curves and $r = 1/\text{h}$. Experimental ranges of the independent variables are as specified for Eq. (7) in Table 3.

3.3. Water activity studies

As with the temperature studies, the growth rate/water activity (NaCl) graph for *P. putida* 1442 (Fig. 2) is representative of the other isolates studied. Fitted parameters are summarised in Table 2.

3.4. The combined temperature/water activity model

McMeekin et al. (1987) found temperature and a_w to act independently on bacterial growth rates and

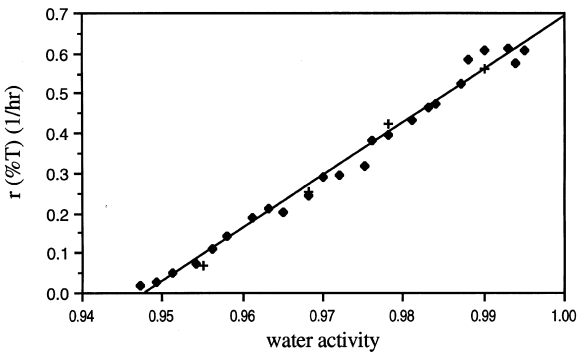

Fig. 2. The growth rate response of *P. putida* 1442 to water activity. $r_{\%T} = 13.339$ ($a_w - 0.948$), $r^2 = 0.987$ for two datasets; one (♦) where $n = 24$ and the other (+) where $n = 4$.

Table 1
Parameters of Eq. (3) (temperature model) for isolates tested in a broth system ($\%T$ data)

Strain	Temperature regime (°C)	T_{min}	b	n‡	r^2#
P. putida 1442	0–30	−7.9	0.0272	26	0.993
	0–15	−7.4	0.0280	29	0.993
Cocktail (5 strains)	0–30	−6.6	0.0270	28	0.990
	0–15	−7.3	0.0282	30	0.990
P. fragi NCIMB 8542	0–30	−6.7	0.0285	26	0.997
	0–30	−7.3	0.0247	30	0.997
P. fragi I6	0–30	−5.0	0.0306	30	0.998
	0–30	−6.1	0.0261	30	0.992
	0–30	−6.1	0.0267	30	0.993
P. fluorescens 1412	0–30	−8.0	0.0252	28	0.993
Mean		−6.8	0.0272		
SD		0.9	0.0002		

‡ is the number of growth curves; # r is the regression coefficient.

Table 2
Parameters of Eq. (5) (water activity model) for isolates (%*T* data)

Strain	$a_{w_{min}}$	b''	n‡	r^2#
P. putida 1442	0.948	13.21	24	0.987
	0.950	14.37	4	0.996
P. putida 1261	0.949	10.44	16	0.992
P. fragi NCIMB 8542	0.951	13.40	14	0.992
P. fluorescens I8.2	0.952	13.29	16	0.973
P. fluorescens 1412	0.955	13.28	15	0.980
Mean	0.951	13.00		
SD	0.002	1.33		

‡ is the number of growth curves; #r is the regression coefficient.

therefore, Eq. (3) could be extended to include a component for water activity by changing b so that

$$b = d\sqrt{a_w - a_{w_{min}}}$$

where d = regression coefficient; a_w = water activity; $a_{w_{min}}$ = theoretical minimum a_w for growth at which growth rate is predicted to be zero. Substituting the term for b into Eq. (3), the new equation for water activity and sub-optimal temperatures becomes

$$\sqrt{r} = d(T - T_{min})\sqrt{a_w - a_{w_{min}}} \quad (6)$$

Similarly, in Eq. (4), b' can also be replaced such that the new equation for water activity and temperature (over the entire biokinetic range) becomes

$$\sqrt{r} = d'(T - T_{min})\{1 - \exp[c(T - T_{max})]\} \sqrt{a_w - a_{w_{min}}} \quad (7)$$

where, in both cases, d or d' is a regression coefficient and all other terms are as previously defined.

Eq. (7) was fitted to *P. putida* 1442 growth rate data by non-linear regression. The fitted model, described in terms of %*T*, is shown below

$$\sqrt{r_{\%T}} = 0.1398\sqrt{a_w - 0.947}(T + 6.04) \{1 - \exp[0.1722(T - 41.17]\}$$
$$n = 113 \qquad \text{RMSE} = 0.0307 \quad (8)$$

where n = the number of growth curves.

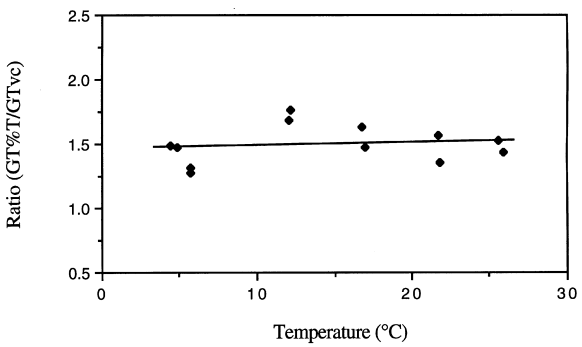

Fig. 3. Ratio ($GT_{\%T}/GT_{VC}$) versus temperature, T (°C) for *P. putida* 1442. $GT_{\%T}/GT_{VC} = 1.4582 + 0.00285\ T$, $r^2 = 0.026$.

3.5. Model calibration

The ratio ($GT_{\%T}/GT_{VC}$) was plotted against temperature (Fig. 3) and shows that there is no trend with respect to temperature. Thus $GT_{\%T}$ can be calibrated to GT_{VC}. Consequently, the parameters (±SE) for the various models for *P. putida* 1442 are described in terms of GT_{VC} (Table 3). The residuals ($\sqrt{r}_{observed} - \sqrt{r}_{predicted}$) were plotted against

Table 3
Parameters (±SE) for the various models for *P. putida* 1442 described in terms of VC[a]

Parameters	Eq. (3)	Eq. (6)	Eq. (4)	Eq. (7)
b, b' d or d'	0.03346±0.0003	0.1539±0.002	0.03772±0.001	0.1709±0.004
c	—	—	0.1719±0.01	0.1723±0.01
T_{min} (°C)	−7.7±0.2	−7.6±0.2	−6.1±0.5	−6.1±0.4
T_{max} (°C)		—	41.2±0.3	41.2±0.2
$a_{w_{min}}$	—	0.947±0.0003	—	0.947±0.0004
Range (°C)	−0.4–28.2	−0.4–28.2	−0.4–39.2	−0.4–39.2
Range (a_w)	0.996	0.947–0.996	0.996	0.947–0.996
n	56	84	85	113
RMSE	0.03346	0.0231	0.0404	0.0372

where $r = 1/h$.

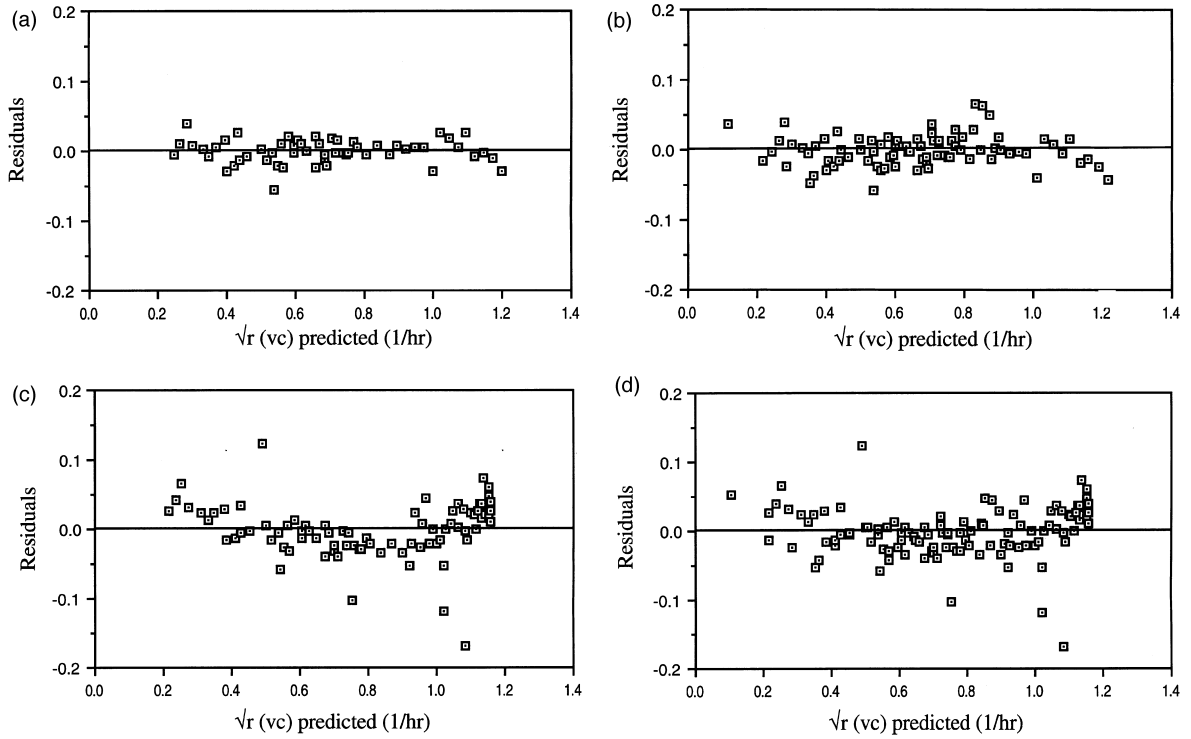

Fig. 4. Residuals ($\sqrt{r}_{observed} - \sqrt{r}_{predicted}$) versus $\sqrt{r}_{predicted}$ for the various models for *P. putida* 1442: (a) the sub-optimal temperature model (Eq. (3)); (b) the combined sub-optimal temperature and water activity model (Eq. (6)); (c) the complete-temperature range model (Eq. (4)) and (d) the combined complete-temperature range and water activity model (Eq. (7)).

$\sqrt{r}_{predicted}$ for the models described by Eqs. (3), (6), (4) and (7) (Fig. 4).

4. Discussion

Determining bacterial growth rates in broth systems using turbidmetric methods provides a rapid and inexpensive means of modelling. The reproducibility of this method is demonstrated in Fig. 1, in which data obtained from two separate experiments are superimposed and provide a slightly better fit ($r^2 = 0.996$) than each dataset treated individually ($r^2 = 0.993$). Of interest are the almost identical average slopes for *P. putida* 1442 and the cocktail, even though one of the slowest strains (*P. fluorescens* 1412) was included in the cocktail. This suggests that the fastest strain rapidly becomes dominant and supports the decision to use the fastest strain for developing a growth rate model adopting a 'worst case' philosophy.

For all water activity models, the observed generation times were shorter than those predicted by the individual model at the lowest water activity; that is, growth occurred below the predicted $a_{w_{min}}$ value. This trend is shown in Fig. 2 but becomes more marked in some of the other strains (figures not shown) and is possibly due to increasing variance in time to growth, as the trend is similar to that observed as temperatures approach the growth/no growth boundary in the temperature experiments. At these extremes there is a possibility for both unusually fast and unusually slow growth. However, often only the fastest rates are detected, as the experiment may be abandoned before growth in the very slow growing cultures is detected. Another explanation may be that the simple straight line model is an approximation only and the true response is more complex. This phenomenon has been discussed in more detail from a theoretical basis in relation to Bělehrádek models for temperature (McMeekin et al., 1993; Chapter 10). Nonetheless, it is unlikely

that psychrotrophic pseudomonads will be the dominant biota under such conditions and thus, the models still have practical utility.

Kamperman (1994) showed *Pseudomonas* growth rate to be unaffected by pH in the range pH 5.4 to 8.6. A pH component has therefore, not been included in the model as most products in which psychrotrophic pseudomonads are the main organism of concern have a pH between 6 and 8. If pH is of concern in a particular product the model would err on the side of safety, as products with a lower pH will have slower growth rates.

Although estimates of GT_{VC} are smaller than $GT_{\%T}$, the difference is constant with respect to temperature ($r^2 = 0.026$ in Fig. 3). Therefore, a simple calibration factor (1.50) for psychrotrophic pseudomonads is able to calibrate $GT_{\%T}$ to GT_{VC} i.e.,

$$GT_{VC} = GT_{\%T}/1.50$$

It is noteworthy that the estimates of the parameters c, T_{min} and T_{max} in Eq. (8) are nearly identical with those of the model only for temperature presented in Section 3.2. The ability to 'embed' a water activity term, with negligible alteration to the rest of the model, is an advantageous feature of the model. An important characteristic of Bělehrádek-type models is that T_{min} is constant for a particular species. This characteristic is important as it simplifies the use of the relative rate concept and the incorporation of models into time temperature function integrators (McMeekin et al., 1993). If models for psychrotrophic pseudomonads are to be considered universal, the T_{min} must remain the same, regardless of the strain of pseudomonad and the food product from which the organism is isolated. Neumeyer (1995) conducted a literature search, divided the data into those from milk and meat (beef, pork and chicken) sources and each dataset was modelled using Bělehrádek-type models (Eq. (3)). The T_{min} estimates are summarised in Table 4. As expected, Neumeyer (1995) noted that as more data was added (i.e. 'n' increased), confidence intervals on the T_{min} estimate decreased. The T_{min} for psychrotrophic pseudomonads is $-7.76°C \pm 0.70$ (95% CI), regardless of the techniques used to determine growth rate or the source of the organism. This implies that only one model to describe the temperature dependence of psychrotrophic pseudomonads is required for a broad range of fresh moist foods, although extra components for other environmental factors (e.g., pH and a_w) may need to be incorporated into the model for particular food products. Similar results are shown in Tables 1 and 2 and the variation in T_{min} and b values were statistically consistent with the isolates being members of one population (Neumeyer, 1995).

Table 4
Summary of T_{min} derived from literature data comparing pseudomonads from dairy origin with those from flesh sources (Neumeyer, 1995)

Pseudomonad source	n‡	Mean T_{min} (°C)	SD	95% CI
Dairy	35	−7.73	2.69	0.89
Flesh	29	−7.80	3.03	1.10
All data	64	−7.76	2.83	0.69

‡ is the number of growth curves.

For *P. putida* 1442 the T_{min} determined by the sub-optimal temperature models (Eq. (3) and Eq. (6)) is $= -7.7°C$ while it is $-6.1°C$ when determined using the complete temperature range models (Eq. (4) and Eq. (7)). The T_{min} for pseudomonads in Table 4 is the same as that for *P. putida* 1442 when both are determined using Eq. (3). Although the variation in T_{min} values, determined using the sub-optimal and complete temperature range models, is minor, it is worth noting because literature comparisons of T_{min} values determined using different equations may require re-evaluation. The differences in T_{min} values are reflected in the RMSE values and in the residual plots of the various models shown in Fig. 4. Importantly, the residual plots for the sub-optimal temperature models (Fig. 4ab) show a balance of positive and negative residuals, while the complete-temperature range models (Fig. 4cd) show a distinctive trend of positive residuals at high and low values of $\sqrt{r}$, predicted with a run of largely negative residuals in the mid-range. Although these results may indicate that the complete-temperature range models are imperfect, it is instructive to compare these figures with a

different equation. Fitting the observed temperature data for *P. putida* 1442 to the equation proposed by Rosso et al. (1993) resulted in a T_{min} of -4.7 ± 0.5°C and a RMSE of 0.0517, i.e. the RMSE is larger than that of the Bělehrádek type models and the difference between T_{min} values determined by the Rosso et al. (1993) model and the complete-temperature range models is greater than the difference between the sub-optimal and full-temperature range Bělehrádek type models.

The trends of the residual and similarities in the RMSE values indicate that it is usually unnecessary to use Eqs. (3) and (4) as the differences in predictions between Eq. (3) and Eq. (6); and Eq. (4) and Eq. (7); are less than that due to measuring error. The differences between Eq. (4) and Eq. (7) are much more pronounced, particularly at refrigeration temperatures. As these are the temperatures of concern in predicting the growth of psychrotrophic pseudomonads, it is proposed that whenever possible (i.e. at sub-optimal temperatures) Eq. (6) should be used to calculate predicted generation times, while Eq. (7) should be used when temperatures are above optimum. Eq. (7) can be used over the entire biokinetic range but it must be accepted that less accurate predictions of generation times may result due to the trend to positive residuals at low temperatures.

The full biokinetic range was studied for completeness, but in practice, pseudomonads only dominate at the lower temperatures and other organisms tend to be responsible for spoilage at temperatures above 15–20°C. The performance of these models is discussed in Neumeyer et al. (1997) and was found to predict accurately the extent of growth of pseudomonads under a variety of situations.

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