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International Journal of Food Microbiology 38 (1997) 133–142

International Journal of Food Microbiology

Development and evaluation of a predictive model for the effect of temperature and water activity on the growth rate of *Vibrio parahaemolyticus*

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Received 11 September 1997; accepted 16 September 1997

Abstract

The growth rates of four strains of *Vibrio parahaemolyticus* were measured and compared in a model broth system. The results for the fastest growing strain, based on 77 combinations of temperature and water activity (a_w) using NaCl as the humectant, were summarised in the form of a predictive mathematical model. The model, of the square-root type includes a novel term to describe the effects of super-optimal water activity, and can be used to predict generation times for the temperature range (8–45°C) and water activity range (0.936–0.995) which permit growth of *Vibrio parahaemolyticus*. Predicted generation times from the model were compared to literature data, using bias and accuracy factors, for both laboratory media and foods. The model was shown to give realistic growth estimates, with a bias value of 1.01, and an accuracy factor of 1.38. © 1997 Elsevier Science B.V.

Keywords: *Vibrio parahaemolyticus*; Predictive microbiology; Growth rate; Temperature; Water activity

1. Introduction

Vibrio parahaemolyticus is a halophilic, gram-negative, food-borne pathogen which multiplies rapidly at room temperature. Generation times as short as ten minutes under optimal conditions have been reported in the literature (Twedt and Novelli, 1971). This organism is a major cause of gastroenteritis in areas where the consumption of raw and semi-processed seafood is common. Common preservation techniques, such as reduction of water activity by drying, smoking or salt curing, successfully inhibit most microorganisms. However, due to it's halophilic nature, *Vibrio parahaemolyticus* can still grow on some of these products. A list of measured and calculated water activities and methods of production of salted and dried seafoods from a range of countries has been compiled (Anon, 1988). The water activity of many of those products is not in the range to exclude the growth of *Vibrio parahaemolyticus*.

In Japan, *Vibrio parahaemolyticus* has been confirmed as the cause of up to 75% of food poisoning

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PII S0168-1605(97)00100-1

outbreaks in the summer months (Okabe, 1974). The distribution of the organism is restricted to inshore estuarine regions and shallow coastal areas. A distinct seasonal cycle has been observed, with a correlation between water temperature and viable counts (Colwell et al., 1984). Numbers are highest in the summer and this is reflected in the seasonal incidence of food poisoning due to the organism. The high incidence in summer is also due to the extremely fast growth rate of *Vibrio parahaemolyticus*. The pathogenicity of *Vibrio parahaemolyticus* has been related to its ability to cause β-haemolysis on a special high-salt medium called Wagatsuma agar, known as the Kanagawa phenomenon.

Predictive microbiology has been used to model the population dynamics of a number of pathogenic and spoilage bacteria of foods. This is possible due to the reproducible nature of a microorganisms response to the environment (McMeekin et al., 1993). Therefore by gathering a detailed knowledge of the growth rate response to the dominant environmental parameters of temperature, a_w and pH, it is possible to predict the extent of microbial proliferation under conditions within the range of experimental values tested. The results are incorporated into a mathematical model, which may enable the evaluation of food safety and remaining shelf life of a food. The value of predictive microbiology becomes evident when it is compared to traditional methods of food safety assessment, which may require lengthy incubation or very high numbers of organisms to be present.

Most attempts at defining the growth of *Vibrio parahaemolyticus* have been qualitative, reporting the presence or absence of growth, rather than the quantitative measure of growth rate undertaken in this study. This organism has been the subject of many scientific publications outlining limits for its growth and survival in various fish products (Matches et al., 1971; Bradshaw et al., 1974; Muntada-Garriga et al., 1995). However, few attempts to formulate a predictive model for this organism, in terms of growth rate under specific conditions, have been published. A model was developed by Lin (1988) to predict the probability of growth initiation, taking into account the effect of temperature, salt concentration and pH. There were found to be no statistically significant two-way interactions between any of these parameters. This supports the observations on microbial growth in general, the effects of these parameters on growth rate being additive, not synergistic (Adams et al., 1991; McMeekin et al., 1993: pp. 188–190). This has been shown by the observation that T_{min} and a_{wmin} (the theoretical minimum values for growth) remain constant under varying conditions, with a_{wmin} dependent upon the humectant used. There may exist synergism between growth-inhibitory factors, however, so that minimum conditions for growth due to temperature, for example, may be modified by reduced water activity (McMeekin et al., 1987). These findings allow the development of a predictive model in much simpler terms, as the effect of each parameter can be determined individually.

This work reports the development of a mathematical model to describe the effect of temperature and water activity on the growth rate of *Vibrio parahaemolyticus* 38.349. The growth response to water activity was modelled with a form of the McMeekin et al. (1987) model which includes a novel term for the effects of super-optimal water activities and which can be used for halophilic organisms. The predicted generation times were compared to observed responses reported in the literature for both laboratory media and food.

2. Materials and methods

2.1. Strains

Four *Vibrio parahaemolyticus* strains were supplied by Dr. P.M. Desmarchelier at the University of Queensland. All were faecal isolates from gastroenteritis cases. Growth rates of all four strains were examined individually, with strain 38.349 exhibiting the fastest growth rate at all conditions of temperature and water activity tested (Temp = 5–50°C; a_w = 0.936–0.995). This strain was used for the development of the model, since it is likely to be indicative of the 'worst-case scenario'.

2.2. Media

Growth experiments were carried out in TSB + S (Tryptone Soya Broth (OXOID) + 3% (w/w) sodium chloride), adjusted to pH 8.0 (within the

optimal pH range for *Vibrio parahaemolyticus*) with NaOH. The broth was sterilised at 121°C for 15 min. For viable counts, agar plates were made by adding 1.5% agar to the TSB + S. Slopes were made in the same way for culture maintenance. Cultures were kept at room temperature and subcultured every two months.

For water activity experiments, the a_w was further adjusted with sodium chloride. Two broths were made, one of a low a_w value, the other high. These were then mixed in appropriate ratios to form a graded series of water activity-modified broths. The values for water activities were calculated from the known amount of salt added to the media, from the water activity values of Resnik and Chirife (1988). These calculations were confirmed by measurement using a dewpoint activity meter (Aqualab Model CX2 - Decagon Devices Inc., Washington, USA).

2.3. Inoculum

A loopful of the maintenance slope culture was transferred to 50 ml of the growth medium in Erlenmeyer flasks. This was incubated statically overnight at 25°C. A second subculture was then made from the first, and incubated at 25°C for 18–24 h prior to beginning the experiment. The broth culture was placed at 10°C for one hour immediately before the commencement of the growth experiment, to lessen the 'cold shock' at lower temperatures. Holding at 10°C also had the effect of slowing the growth rate of the inoculum which ensured minimal changes in cell density during the inoculation procedure.

2.4. Experimental procedure

For all growth rate determinations a temperature gradient incubator (TGI - Model TN 3, Advantec, Toyo Roshi International, USA) was used. Aliquots of 15 ml of the growth medium were aseptically dispensed into sterile L-shaped glass test tubes. The tubes were left overnight to achieve the appropriate temperature and then the inoculum was added. For temperature experiments the gradient was set from 0–50°C, with 30 tubes at approximately 1.5°C intervals. Water activity experiments were conducted at a constant 20±0.5°C. Growth was assessed turbidimetrically at 540 nm. In a separate experiment, growth was monitored at three temperatures by both turbidimetry and viable count. For the latter, 0.1 ml samples of cultures were removed at set times. Decimal dilutions were prepared in 0.1% peptone water plus 3% salt, and 0.1 ml of three appropriate dilutions were spread on TSA + S plates and incubated at 25°C. Plates which contained between 30 and 300 colonies were counted manually. From this, the number of cells in the sample was calculated, by the method of Farmiloe et al. (1954). Generation times for each data set were estimated by use of a Gompertz function as described in McMeekin et al. (1993); (pp. 84–86).

Dalgaard et al. (1994) have shown that turbidimetric methods are as precise as viable counts and, because they are much less labour intensive and faster than plate counting, are useful for predictive microbiology. However, an inherent difference exists between estimates of the generation time determined by turbidimetric or viable count methods. A constant ratio between the two techniques was reported by Dalgaard et al. (1994), who demonstrated viable count methods provide lower estimates of generation time. This was found to apply over the whole temperature range, and appears to be a systematic error of the turbidimetric method for assessing microbial growth (Ross, 1993; Dalgaard et al., 1994). The two methods of growth rate determination have been compared for *Vibrio parahaemolyticus* as a ratio Generation time (%T): Generation time (VC) and were found to be 1.41±0.13, the average of duplicate determinations at 13, 26 and 39°C (Miles, 1994). This factor was included in parameter *b* of the final model.

2.5. Construction of the model

Modelling was carried out in two stages. The first stage involved modelling the bacterial growth curves using a Gompertz function. The Gompertz function has been reported to underestimate generation times by ~13% (Baranyi et al., 1993; Ross, 1993). This factor was also included into parameter *b* of the final model, so that the final model presented predicts the generation time that would be estimated by drawing a tangent to the steepest slope of a growth curve based on viable count measurements as has traditionally been done. This calibration factor was also taken into account for literature data, where it was stated

that data was fitted with the Gompertz function, or where it had been fitted by the authors to data gained from graphs.

In the second stage growth rate was modelled as a function of temperature and water activity. The generation times were converted to their reciprocal, the growth rate k, and the square root of k was calculated for model fitting to homogenise the variance in growth rate estimates (Zwietering et al., 1994). The four parameter square root model (Ratkowsky et al., 1983) was fitted to $\sqrt{}$(growth rate) data as a function of temperature. An extended water activity model (Eq. (1)), similar in structure to the four parameter square root model for temperature, was used to model water activity limited growth rate responses.

$$\sqrt{k} = b\sqrt{(a_w - a_{wmin})\{1 - \exp[d(a_w - a_{wmax})]\}} \quad (1)$$

where:

k = growth rate
a_{wmin}, a_{wmax} = theoretical lower and upper water activity limits b and d are parameters fitted by non-linear regression

This model is an extension of the two-parameter square root model for water activity first proposed by McMeekin et al. (1987). The water activity term was combined with the four parameter square root model to form Eq. (2):

$$\sqrt{k} = b(T - T_{min})\{1 - \exp[c(T - T_{max})]\} \cdot\sqrt{(a_w - a_{wmin})\{1 - \exp[d(aw - a_{wmax})]\}} \quad (2)$$

where:

T = temperature
T_{min} = the lower temperature at which the fitted equation equals zero
T_{max} = the upper temperature at which the fitted equation equals zero
b, c = are coefficients to be fitted
and all other terms are as previously defined

The parameters for the combined model for temperature and water activity were estimated by nonlinear regression using PROC NONLIN of SAS (Statistical Analysis System, SAS Institute Incorporated, USA). Conditions where growth was not observed were not included in the development of this model.

3. Results and discussion

3.1. Effect of temperature on growth rate

The minimum observed temperature for growth was 8.3°C, while the maximum temperature for growth was observed at 45.3°C, the optimum occurring between 37–39°C. T_{min} for the fastest growing strain, 38.349, was calculated to be 5.3±0.3°C. In most cases at the extreme temperatures the final population density did not appear to be as high as at moderate temperatures. The minimum observed temperature for growth may be altered by changing the composition of the growth medium, but T_{min} remains constant, as it is apparently an intrinsic property of the organism. However, there does appear to be some minor strain to strain variation (see Table 1), with T_{min} values ranging from 5.3°C to 7.6°C for the four strains of *Vibrio parahaemolyticus* used in these experiments.

3.2. Effect of water activity on growth rate

Vibrio parahaemolyticus strains grew over the water activity range 0.936 to 0.995 (9.6–0.4% NaCl) with an optimum between 0.982 and 0.987. Growth at a_w 0.995 was slower than in the optimal range, with a_{wmax} estimated to be between 0.997 and 0.999. The fit of Eq. (1) to water activity data at a constant temperature of 20°C is shown in Fig. 1. Growth was observed at a_w 0.936 (9.6% NaCl), the lowest value tested. This is much lower than the previously reported minimum water activity for growth of 0.945 (8.4% NaCl) (Beuchat, 1974). It was stated by van den Broek et al. (1979) that the classical identification experiment of no growth at 10% salt is not

Table 1
Parameter values for all strains of *Vibrio parahaemolyticus* tested

Strain no	T_{min}	T_{max}	a_{wmin}	a_{wmax}
38.349	278.5	319.6	0.921	0.998
38.320	279.9	317.3	0.926	0.997
38.317	280.7	316.5	0.939	0.999
38.309	278.1	321.0	N/A	N/A

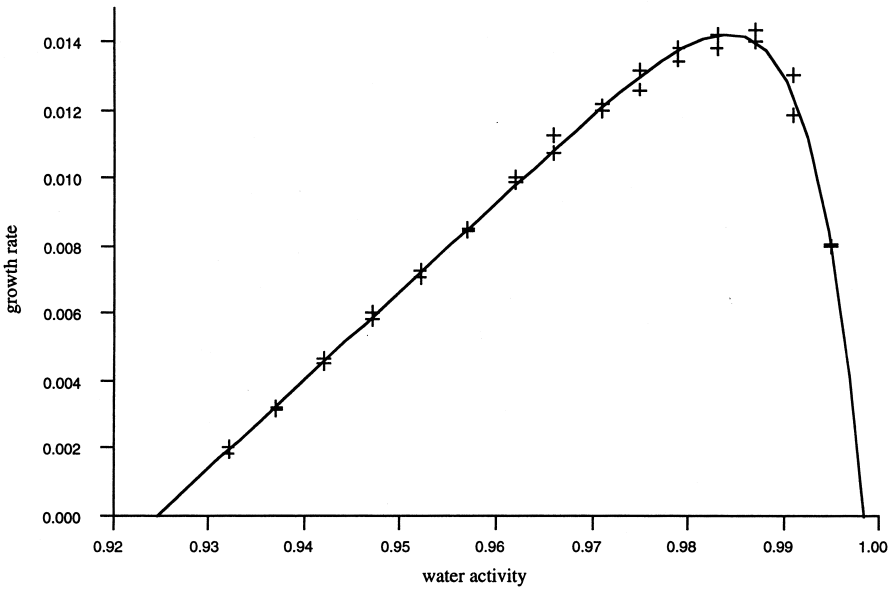

Fig. 1. Comparison of four parameter water activity model (Eq. (1)) and experimental data at 20°C for *Vibrio parahaemolyticus* strain 38.349.

reliable, and Twedt et al. (1969) reported variable growth of *Vibrio parahaemolyticus* at 10% salt (a_w 0.933) and infrequent growth at 13% salt (a_w 0.909), with eight out of 79 strains being positive. As stated by Sperber (1983) the conflicting values reported in the literature may be indicative of defects in the methods used to determine water activity and illustrates the need to standardise methodology.

3.3. The model

Square root models have been developed to model growth rates under conditions where growth is possible. They are not intended to model conditions where growth does not occur, although a separate approach for this based on square root type models has been described (Ratkowsky and Ross, 1995). Accordingly, conditions where growth was not observed were not included in the development of this model. In general, models excluding no-growth data yield a better fit to experimental data. Inclusion of no-growth data has been attempted in the past (Buchanan and Phillips, 1990), through the use of a transformation of the Gompertz parameter M to $1/M$, but this process did not enhance the accuracy of the models, and it was not included here. Inclusion of no-growth data in the development of models may bias the equation (Buchanan and Phillips, 1990). Similarly, the model is for growth rate after the resolution of lag time. While the model does not explicitly consider lag time, Baranyi and Roberts (1994) noted that lag time response to environmental conditions is often proportionally the same as the generation time response, i.e. conditions which double the generation time will also double the lag time, conditions which reduce generation time four fold will also reduce the lag time four fold.

3.4. Range of applicability

The model was based solely on observations made of strain 38.349, as this was the fastest growing of four strains tested under all conditions of water activity and temperature measured. Use of the data for the fastest growing strain ensures the model does not underpredict the growth rate (i.e. predict a slower growth rate than is actually observed). Ideally the model should overpredict and be on the 'fail-safe' side of prediction, but not to the point where its predictions are not close to the observed growth rate. A total of 77 growth curves were used in the development of the model, temperature range 8.3 to

45.3°C and water activity range 0.936 (9.6% NaCl) to 0.995 (0.5% NaCl).

The parameter pH has not been included in this model, as growth rate was shown to be relatively constant over the pH range 6.5–8.9 (Miles, 1994). Included in this pH range, and thus applicable to the model, are most fish species, crabs, prawns, shrimps and clams. Organisms rich in glycogen may have a lower ultimate pH due to lactic acid production. Application of the equations of Presser et al. (1997) for the effect of lactic acid in the range 20 to 200 mM showed the pH_{min} to be 5.42±0.04, but the effect of a combination of pH and salt, or other humectants, has not yet been studied. The interpolation region (Baranyi et al., 1996) for this data is shown in Fig. 2. Note that as this model contains only two variables the region is, by analogy to the minimum convex polyhedron, the minimum polygon which encloses the interpolation space. More specifically, because of the experimental design, the interpolation region is a tetragon.

The range of data needs to be wide because of the danger of extrapolating beyond the limits of the model, particularly for empirical models. Also, the more points that are able to be included in the model, the less is the emphasis placed on any particular point. Although the growth rate of the organism was relatively constant over the pH range 6.5 to 8.9, further work is required on the effects of pH on growth rate in the range 6.5 to 5.0, the minimum pH at which growth was observed, and below which the organism dies (Vanderzant and Nickelson, 1972; Beuchat, 1973; Miles, 1994). The final model for the combined effects of water activity and temperature on the growth rate (k) of *Vibrio parahaemolyticus* in the pH range 6.5 to 8.9 is shown in Eq. (3).

$$\sqrt{k} = 0.035634(T - 278.5)\{1 - \exp[0.3403(T - 319.6)]\} \cdot\sqrt{(a_w - 0.921)\{1 - \exp[263.64(a_w - 0.998)]\}} \quad (3)$$

Root Mean Square Error (RMSE) = 0.00595

k = growth rate (min^{-1}), calibrated to viable count results
T = temperature in K in the range 281.8–328.5
a_w = water activity in the range 0.936–0.998

This model has been calibrated (see Section 2) to produce estimates consistent with those that would be obtained from the slope of a tangent drawn to the

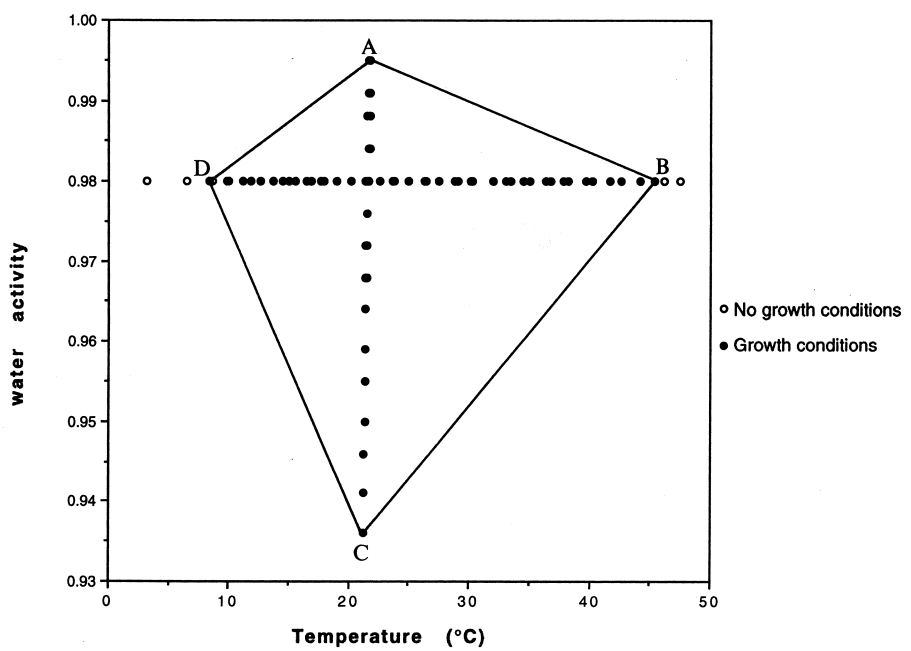

Fig. 2. Experimental conditions tested to generate the model and illustrating the interpolation region (area enclosed by the tetragon ABCD).

steepest part of the exponential phase of the growth curve based on viable count data.

3.5. Comparison of model predictions with literature data

Table 2 shows the eight data sets containing 121 generation times reported for *Vibrio parahaemolyticus* obtained from the literature. Comparison with literature data is often difficult because assumptions have to be made. Authors often do not state the exact conditions under which the experiments were conducted, deficiencies which have been highlighted in the past (McClure et al., 1994). On many occasions the a_w value was either not stated, or appeared to be incorrectly calculated. In other cases a graph was presented but no generation time data was shown. In these cases the values were calculated manually from an enlarged copy of the graph. Assumptions made with the data sets are listed in Table 2.

Bias and accuracy factors (Ross, 1996) are listed in Table 2 for each individual data set and for all the individual data points combined. These values provide a guide to the overall performance of the model. The bias factor assesses whether, the model is 'fail-safe', while the accuracy factor gives an averaged measure of how close predictions are to observations. The bias factor was calculated to be 1.01; suggesting the model is neither over or under predicting the growth rate. The accuracy factor has a value of 1.38. This indicates that, on average the observed generation time is within 38% of the predicted generation time. A perfect fit is indicated by a value of 1, the more the factor is above this value, the less precise is the average estimate. Bias and accuracy factors can also be used to demonstrate the quality of the data sets used for comparison. The values for the Barrow and Miller (1976) data, and the accuracy values for some of the other data sets, are quite high and tend to suggest that some of the assumptions may have been incorrect. Evaluation of the model with more data of higher quality may help to improve the overall fit of the model. Considering the assumptions which had to be made with the literature data the performance of the model appears to be quite good.

From Table 2 it is clear that even though *Vibrio parahaemolyticus* 38.349 was the fastest growing of four strains examined, faster growing strains have been reported. Table 2 also suggests that there is wide variation in growth rates of strains of *Vibrio parahaemolyticus*., or alternatively that there is variation in the reliability of published growth rate estimates. Fig. 3 shows the comparison of predicted values from the model with observations from published data. The middle diagonal line represents perfect agreement between observed and predicted values (ie. line of equivalence). It can be seen that most points fall about this line, thus indicating the model is predicting realistic generation times and is not necessarily under-predicting to give 'fail-safe' estimates. The other two lines represent the accuracy levels ($\pm$38%) and it can be seen how the majority

Table 2
Bias and accuracy factors for individual data sets, and overall model performance

Reference	*n*	Temp range	a_w range	Media	Assumptions[a]	Bias	Accuracy
Jackson, 1974	30	8–42°C	0.981	TSB + 2.5% NaCl	2, 3, 4, 5	1.08	1.42
Barrow and Miller, 1976	6	37	0.995	Prawn, crab, cod, meat	1, 5	1.88	1.88
Nelson and Potter, 1976	19	25–37	0.983–0.997	Nutrient broth, egg, pudding, milk, turkey, beef	1, 2	1.07	1.62
Bradshaw et al., 1984	8	25–35	0.995	Homogenates of shrimp, crab, oyster	1, 5	0.89	1.35
Ulitzur, 1974	11	37	0.981	SWYP	1	1.09	1.18
Beuchat, 1974	6	29	0.949–0.995	TSB + added NaCl	2, 4	0.96	1.31
Twedt and Novelli, 1971	37	35	0.981	Peptone-based, other	1, 3	0.83	1.29
Pace and Chai, 1989	4	35	0.995	PPBE + 0.5% NaCl, PPBE + 10.1% NaCl		1.21	1.21
Overall Model		8–45°C	0.936–0.998			1.01	1.38

[a] Assumptions: 1. Assumed water activity values; 2. Values calculated from graph; 3. Turbidity converted to viable count; 4. Water activity calculated from % NaCl; 5. Adjusted for Gompertz ($\times$1.13).

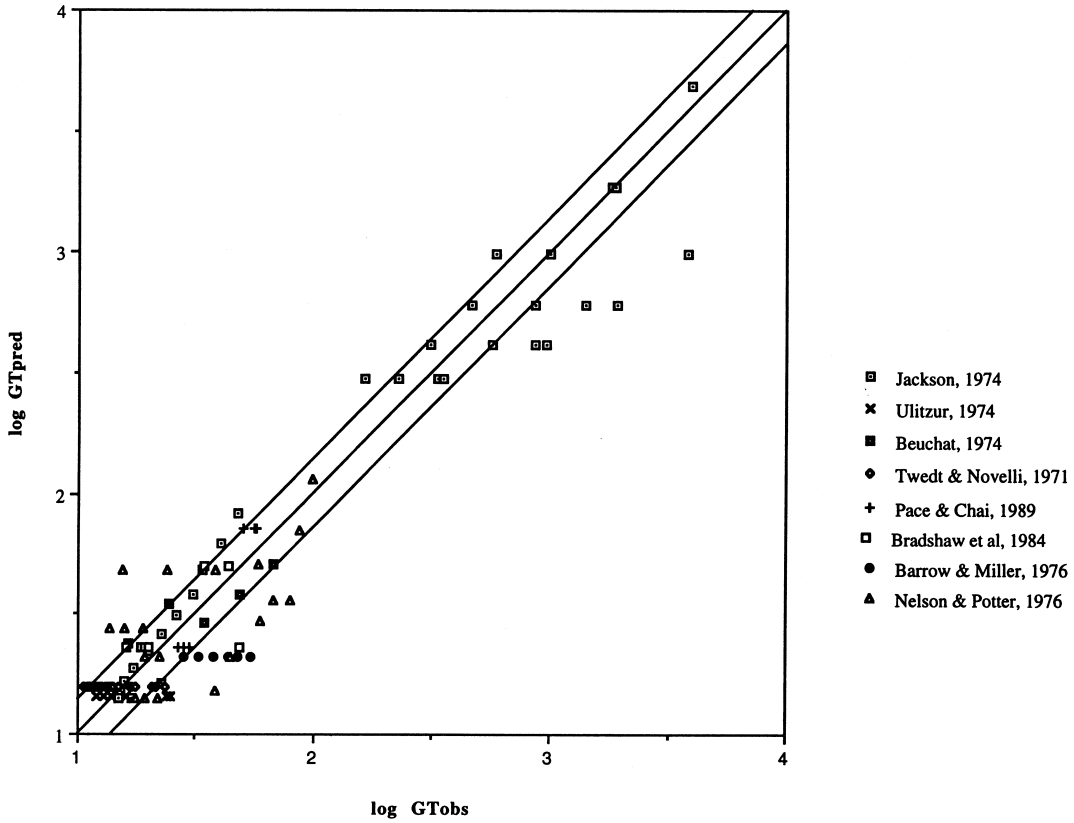

Fig. 3. Comparison of model predictions with literature data. Middle diagonal line is where predicted = observed (line of equivalence), points below this line are 'fail-safe'. The other two lines represent the accuracy factor of 38%.

of the literature data sets fall within these levels. The plot of residuals (Fig. 4) for the model predictions against the literature data shows the distribution of data is not biased. Therefore the model gives realistic predictions, neither systematically over- or under predicting.

4. Conclusions

A model for estimating the growth rate of *Vibrio parahaemolyticus* has been developed and its performance objectively evaluated. Considering the assumptions which were necessary to derive independent data from the literature, the model's predictions agree well with those independent observations, and the model's performance is consistent with the range of performance that can be expected from predictive models when compared to literature data (Ross, 1996). The model is applicable over the entire range of conditions for temperature and water activity which permit growth of *Vibrio parahaemolyticus* 38.349, and according to measures of bias and accuracy the model gives unbiased predictions and is neither systematically under or over predicting generation times.

Acknowledgements

This research was funded through the Australian Research Council (ARC) small grants scheme. The strains of *Vibrio parahaemolyticus* were provided by Dr. P.M. Desmarchelier, CSIRO Division Food Science and Technology, Brisbane Laboratory. David Ratkowsky is thanked for his help with his statistical expertise and application during the preparation of this manuscript.

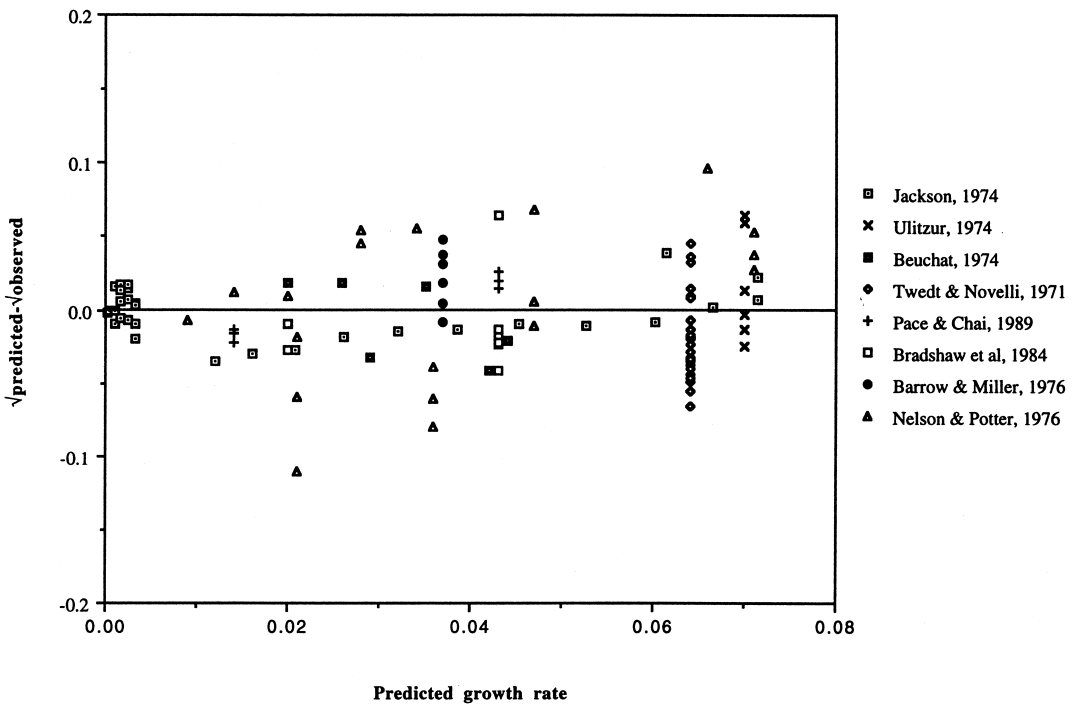

Fig. 4. Residual plot of predicted growth rate against literature data. The scatter of data shows no systematic over or under prediction by the model.

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