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Evaluation of Data Transformations and Validation of a Model for the Effect of Temperature on Bacterial Growth

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The temperature of chilled foods is an important variable for controlling microbial growth in a production and distribution chain. Therefore, it is essential to model growth as a function of temperature in order to predict the number of organisms as a function of temperature and time. This article deals with the correct variance-stabilizing transformation of the growth parameters A (asymptotic level), μ (specific growth rate), and λ (lag time). This is of importance for the regression analysis of the data. A previously gathered data set and model for the effect of temperature on the growth of *Lactobacillus plantarum* (M. H. Zwietering, J. T. de Koos, B. E. Hasenack, J. C. de Wit, and K. van 't Riet, Appl. Environ. Microbiol. 57:1094–1101, 1991) is extended with new data. With the total data set (original and new data), a variance-stabilizing transformation is selected in order to determine which transformation should precede fitting. No transformation for the asymptote data, a square root for the growth rate, and a logarithmic transformation for the lag time were found to be appropriate. After these transformations, no significant correlation was found between the variance and the magnitude of the variable. Model corrections were made and model parameters were estimated by using the original data. With the new data, the models were validated by comparing the lack of fit of the models with the measurement error, using an F test. The predictions of the models for μ and λ were adequate. The model for A showed a systematic deviation, and therefore a new model for A is proposed.

Temperature is a major factor determining the progress of many food deterioration reactions. For microbial spoilage, the effect of temperature on the specific growth rate (μ) and the lag phase (λ) is important. Various models are used to describe the effect of temperature (6). Models often are compared only with the data on which the model is fitted (measured versus fitted) and are only rarely validated with new data (measured versus predicted). Yet such validation can provide useful information about the accuracy and predictive value of the models.

The effect of temperature on growth rate is often modeled after a transformation (square root or logarithm). This transformation, however, changes the distribution of errors. Unweighted regression may only be performed if the variance is independent of the magnitude of the growth rate. Therefore, it is of great importance to determine which type of transformation gives a constant variance. Ratkowsky (3) used multiple measurements at each temperature to calculate the variance. He advises the use of a square root transformation to stabilize variance for the growth rate (or [generation time]$^{-0.5}$) and a logarithm for the lag phase duration. Alber and Schaffner (1) used the in-experiment error (no replicates) to calculate the variance and recommended the use of a logarithmic transformation to stabilize the variance of the growth rate. In our earlier article (6), the growth rate and the asymptote (A) were modeled without transformation because the variance seemed to be equally distributed in that particular data set. The lag time was fitted after a logarithmic transformation, since this transformation stabilized the variance.

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It is clear that different opinions exist. In order to estimate the variance, at least duplicate measurements at each temperature are needed. With an extensive data set, which transformations stabilize the variances of A, μ_m, and λ over a large range were examined.

First, variance-stabilizing transformations were selected for A, μ_m, and λ (independent at any model). After transformation of the data, models for the effect of temperature on A, μ_m, and λ were fitted. With a new set of data, these models were tested. Finally, all data together were used to update the models.

THEORY

Description of experimental bacterial growth rate data. Growth curves are defined as the logarithm of the relative population size [ln (N/N_0)] as a function of time. A sigmoidal growth model (modified Gompertz) with three parameters can describe the growth curve (7) at a given temperature:

$$\ln (N/N_0) = A \exp \left\{ -\exp\left[\frac{\mu_m \cdot e}{A}(\lambda - t) + 1 \right] \right\} \quad (1)$$

where A is the asymptotic level $\ln(N_\infty/N_0)$, μ_m is the maximum specific growth rate (per hour), λ is the lag phase duration (hours), t is time (hours), and e is exp(1).

Selection of variance-stabilizing transformations. In order to determine which transformation should precede fitting, variance-stabilizing transformations must be selected. At one temperature, T_i, a total number, m_i, of replicate curves are measured. In our case, m_i does not have the same value at different temperatures. The variances of A, μ_m, and λ are calculated at each temperature by using the mean values of the measured data. Then the model for the best prediction of the y value (A, μ, or λ) at a certain temperature can be proposed that is defined as the mean value $\bar{y}(i)$ of the

TABLE 1. Parameter values and models for the effect of temperature (T) on the asymptote (A), growth rate (μ_m), and lag time (λ) for *L. plantarum* in MRS medium[a]

Parameter	Model and equation	Variable	Value
Asymptote	Ratkowsky 4, $A = b_4 \{1 - \exp [c_4(T - T_{max4})]\}$	b_4 (−)	8.46[b]
		c_4 (°C^{-1})	1.25
		T_{max4} (°C)	43.1
Growth rate	Ratkowsky 3, $\mu_m = [b_3 (T - T_{min3})]^2 \{1 - \exp [c_3 (T - T_{max3})]\}$	b_3 (°C^{-1} h$^{-0.5}$)	0.0410
		T_{min3} (°C)	3.99
		c_3 (°C^{-1})	0.161
		T_{max3} (°C)	43.7
Lag time	Hyperbola,[c] $\ln (\lambda) = \frac{p}{(T - q)}$	p (h)	23.9
		q (°C)	2.28

[a] Data are from Zwietering et al. (6).
[b] Parameter b_4 depends on the inoculation level according to the equation $b_4 = 21.58 - \ln (N_0)$.
[c] p is a measure for the decrease in the lag time with temperature, and q is the temperature at which the lag time is infinite (no growth).

measured y values at that temperature. This model is called the general model:

$$\bar{y}(i) = \sum_{j=1}^{m_i} \frac{y(i, j)}{m_i} \qquad (2)$$

where y is A, μ, or λ; $y(i,j)$ is the j^{th} y value at T_i, and $\bar{y}(i)$ is the mean y value at T_i.

The variance at temperature T_i is calculated at temperatures for which more than one observation is obtained, with:

$$\mathrm{RSS}_i = \sum_{j=1}^{m_i} [y(i, j) - \bar{y}(i)]^2$$
$$s_i^2 = \frac{\mathrm{RSS}_i}{\mathrm{DF}_i} \qquad (3)$$

where RSS_i is the residual sum of squares at T_i, DF_i is the degrees of freedom at T_i (equals $m_i - 1$), and s_i^2 is the residual variance at T_i.

According to Ratkowsky (3), the variance of A, μ_m, and λ can be plotted against the mean value as well as the variance divided by the mean, the square of the mean, and the cube of the mean in order to determine the appropriate transformation. If the variance is dependent on the mean, models should be fitted after transforming the data or by using nonnormal error assumptions. If the variance divided by the mean shows no correlation, a square root transformation is suitable [$\mathrm{var}(\sqrt{y_i}) = \mathrm{var}(y_i)/4y_i$]. If the variance divided by the square of the mean shows no correlation, a logarithmic transformation is suitable to correct for heterogeneity of variance [$\mathrm{var}(\ln [y_i]) = \mathrm{var}(y_i)/y_i^2$].

As an alternative procedure, the variances of A, μ_m, and λ are calculated after carrying out the transformation (the variance of the transformed data). Then the variances of the untransformed data, of the square root, and of the logarithm of the data are plotted against the mean.

To quantify correlation (for both of the abovementioned methods), linear regression is carried out and the correlation coefficient is calculated. With Student's t test, it can be examined whether there is a correlation:

$$t_{stud} = \frac{r\sqrt{n - 2}}{\sqrt{1 - r^2}} \qquad (4)$$

where t_{stud} is Student's t value, r is the correlation coefficient, and n is the number of observations.

It should be noted that linear regression is used, although the relations will not be linear. This gives a global indication of the correlation and not an exact value. If there is no linear correlation, this does not mean that there is no other correlation. Visual inspection of the variance data is also crucial.

Growth-temperature relations. After transforming the A, μ_m, and λ data, the effect of temperature on these variables can be modeled. The previously proposed models for the

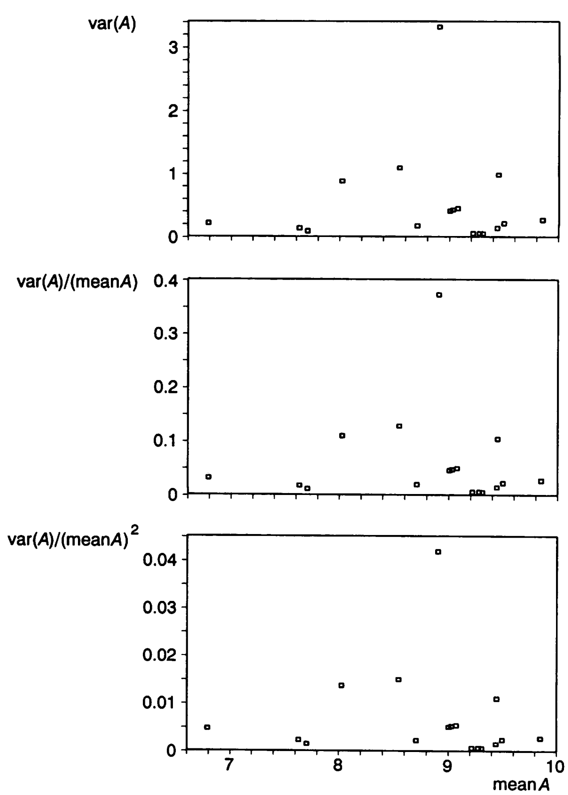

FIG. 1. Variance (var), of A, variance of A divided by the mean, and variance of A divided by the square of the mean, plotted against the mean of A.

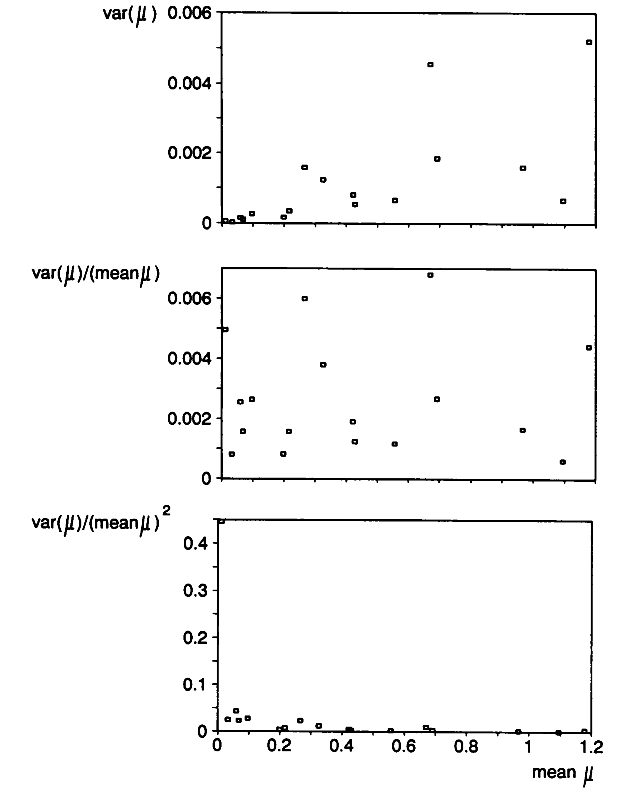

FIG. 2. Variance (var) of μ, variance of μ divided by the mean, and variance of μ divided by the square of the mean, plotted against the mean of μ.

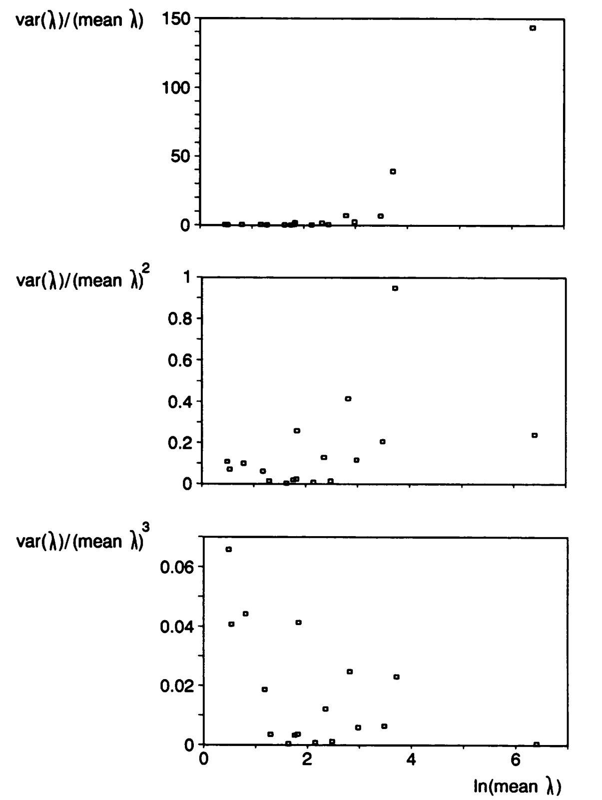

FIG. 3. Variance (var) of λ divided by the mean and divided by the square and the cube of the mean, plotted against the logarithm of the mean of λ.

effect of temperature on the asymptote (A), growth rate (μ_m), and lag time (λ) with parameter values are given in Table 1.

The hyperbolic model for the lag time has the complication that the lag time at higher temperature approaches asymptotically to 1 (the logarithm of the lag time approaches zero). This is of course an arbitrary value, and it is independent of the unit in which the lag time is expressed. This is an undesirable imperfection of the hyperbolic model. Furthermore, it can be assumed that the lag phase increases at temperatures higher than the optimum, which can also be seen in the data. The hyperbolic model, however, does not show such behavior. Therefore, the previously proposed reciprocal of the Ratkowsky model (equation 6) is reconsidered, since this model overcomes these two problems. However, this model contains four parameters. This can be overcome by assuming that the T_{min5} and T_{max5} values, and possibly also the c_5 value, are equal to the parameters of the equation describing the growth rate (T_{min2}, T_{max2}, and c_2; equation 5),

$$\mu = \left(b_2(T - T_{min2}) \cdot \{1 - \exp[c_2(T - T_{max2})]\}\right)^2 \qquad (5)$$

$$\ln(\lambda) = \ln\left[\left(b_5(T - T_{min5}) \cdot \{1 - \exp[c_5(T - T_{max5})]\}\right)^{-2}\right] \qquad (6)$$

where b_2, c_2, b_5, and c_5 are regression coefficients, T_{min2} and T_{max2} are the minimal and maximal temperatures for the growth rate, respectively, and T_{min5} and T_{max5} are the minimal and maximal temperatures for the lag time, respectively.

Comparison of the models. The models are validated statistically with the use of the F ratio test. With the general model (equation 2), the measurement error is estimated by determining the deviation of the measured values from the mean value at one temperature. The cumulative sum of squares of the deviations between the data and the general model is calculated for all temperatures (RSS_g):

$$RSS_g = \sum_{i=1}^{k} RSS_i \qquad (7)$$

$$= \sum_{i=1}^{k} \sum_{j=1}^{m_i} [y(i,j) - \bar{y}(i)]^2 \quad \text{(general model)}$$

where $y(i, j)$ is the j^{th} y value at T_i, $\bar{y}(i)$ is the mean y value at T_i, and k is the total number of different temperatures measured.

The sum of squares of the deviations between the data and the value predicted by a particular growth-temperature model (RSS_m) is calculated as:

$$RSS_m = \sum_{i=1}^{k} \sum_{j=1}^{m_i} [y(i,j) - \hat{y}(i)]^2$$

$$\text{(growth-temperature model)} \qquad (8)$$

where $\hat{y}(i)$ is the model prediction at temperature T_i.

TABLE 2. Results of determination of the correlation coefficient by performing linear regression of the variance data as a function of the mean of the data over the suboptimum temperature range

Parameter	Ratkowsky procedure (3)			Alternative procedure		
	Test[a]	r[b]	t_{stud} value[c]	Transformation	r	t_{stud} value
A	Var	0.0305	**0.118**	None	0.0305	**0.118**
	Var/mean	−0.00440	**−0.0171**	$\sqrt{A}$	−0.0178	**−0.0689**
	Var/mean^2	−0.0442	**−0.171**	ln (A)	−0.0686	**−0.266**
	Var/mean^3	−0.0900	**−0.350**	$1/\sqrt{A}$	0.123	**0.478**
μ	Var	0.672	3.51	None	0.672	3.51
	Var/mean	0.0375	**0.145**	$\sqrt{\mu}$	0.0176	**0.0681**
	Var/mean^2	−0.357	**−1.48**	ln (μ)	−0.671	−3.51
	Var/mean^3	−0.299	**−1.22**	$1/\sqrt{\mu}$	0.848	6.19
λ	Var	0.998	60.9	None	0.998	60.9
	Var/mean	0.978	18.1	$\sqrt{\lambda}$	0.975	17.0
	Var/mean^2	0.146	**0.573**	ln (λ)	0.331	**1.36**
	Var/mean^3	−0.238	**−0.949**	$1/\sqrt{\lambda}$	0.321	**1.31**

[a] Var, variance; var/mean, variance divided by the mean; var/mean^2, variance divided by the square of the mean; var/mean^3, variance divided by the cube of the mean.
[b] Correlation coefficient.
[c] The 95% critical Student's t test value for 15 degrees of freedom is 2.13. Values in boldface type indicate no significant correlation.

RSS_m will always be larger than RSS_g. The RSS_m of the growth-temperature model consists of both the measurement error and the lack of fit; therefore, the difference between the RSS_m of the model and the RSS_g (the sum of squares due to the measurement error) is calculated as an estimate of the lack of fit. If the mean square of the lack of fit [$(RSS_m - RSS_g)/(DF_m - DF_g)$] is of the same order of magnitude as the mean square of the measurement error (MS_{error}), the model is adequate. This comparison between the lack of fit and the measurement error can be quantified statistically by the f test value:

$$f = \frac{(RSS_m - RSS_g)/(DF_m - DF_g)}{MS_{error}} \quad \text{tested against } F_{DF_{error}}^{DF_m - DF_g} \qquad (9)$$

where DF_g is the number of degrees of freedom due to the residual variance, which equals the total number of observations minus the number of different temperatures measured; DF_m is the number of degrees of freedom from the growth-temperature model that equals the number of observations minus the number of estimated parameters; and MS_{error} is the mean square of the measurement error with DF_{error} degrees of freedom.

MATERIALS AND METHODS

Microbial experiments. In 60 experiments at 17 different temperatures, *Lactobacillus plantarum* (American Type Culture Collection [ATCC] determined; no ATCC number) was cultivated in MRS medium (Difco Laboratories). The culture was stored frozen (−16°C). The bacteria were cultivated twice at 30°C, for 24 h and for 16 h. These preincubations were performed to get the organisms in a reproducible condition. Growth was monitored in 20-ml tubes, each containing 10 ml of MRS medium and inoculated with the (preincubated) test organism to reach a target initial titer of

TABLE 3. Parameter estimates of the Ratkowsky model for the $\sqrt{\mu}$ data

Parameter	Estimate	95% Confidence interval	Model
b_2 (°C^{-1} h$^{-0.5}$)	0.0385	0.0343–0.0427	$\sqrt{\mu_m} = b_2(T - T_{min2})$
T_{min2} (°C)	3.37	1.60–5.13	$\{1 - \exp[c_2(T - T_{max2})]\}$
c_2 (°C^{-1})	0.256	0.175–0.336	
T_{max2} (°C)	44.7	44.1–45.4	

5×10^5 CFU/ml. The test tubes were incubated statically at different temperatures from 6 up to 40°C as follows (temperatures in degrees centigrade and number of experiments in parentheses): 6.0 (1); 8.9 (1); 9.8 (7); 10.0 (5); 11.9 (1); 14.0 (1); 14.9 (6); 15.2 (5); 16.7 (3); 18.2 (1); 19.8 (6); 20.2 (7); 24.8 (7); 25.0 (6); 30.0 (1); 34.9 (1); and 40.8 (1). At appropriate time intervals (depending on the temperature), the inoculated cultures were vortexed, and 0.1-ml samples were removed for serial dilution in sterile peptone saline solution (1 g of Bacto-Peptone [Difco] and 8.5 g of NaCl [Merck p.a.] per liter). The number of bacteria was determined on a pour plate (MRS medium with 12 g of agar [Agar Technical, Oxoid Ltd.] per liter). The pour plates were incubated for 48 h at 30°C before bacteria were counted.

Fitting of data. The model equations were fitted to the data by nonlinear regression with a Marquardt algorithm (6, 7).

RESULTS AND DISCUSSION

Selection of variance-stabilizing transformations. The previously measured data set (38 growth curves, from Zwietering et al. [6]) was extended with new data (60 growth curves). Of this total data set (98 growth curves), only the subset of growth parameters (A, μ, and λ) up to 35°C (80 growth curves at 17 different temperatures) were used to determine the variance at different temperatures. This subset is used because, above the optimum temperature (35°C), the variances are much larger than below the optimum, since growth decreases rapidly, resulting in large errors. These variances cannot be reduced by carrying out a transformation. With this subset, the variance is analyzed to find which transformations for A, μ, and λ are necessary: none, a square root, or a logarithm. The variance at different temperatures as a function of the mean value of the variable is given in Fig. 1, 2, and 3. Furthermore, the variances divided by the mean, by the square of the mean, and by the cube of the mean are given, according to the procedure proposed by Ratkowsky (3). For these data, the correlation coefficient was determined by performing a linear regression of the variance data. With Student's t test, it was determined whether correlation was significant. The results are given in Table 2.

TABLE 4. Statistical test of the Ratkowsky model for the $\sqrt{\mu}$ data[a]

Model	No. of parameters	DF	RSS	MS	f	F
Ratkowsky	4	34	0.125	0.00367		
LOF		14	0.023	0.00162	0.317	2.2
General	18	20	0.102	0.00511		

[a] LOF, lack of fit; DF, degrees of freedom; RSS, residual sum of squares; MS, mean square; f, $MS_{LOF}/MS_{general}$; F, F table value (95% confidence).

TABLE 5. Parameter estimates for the ln (λ) models

Parameter[a]	Estimate	95% Confidence interval	Model
p (h)	23.9	19.1–28.7	$\ln(\lambda) = \frac{p}{(T-q)}$ (model 1)
q (°C)	2.28	1.19–3.37	
b_5 (°C^{-1} h$^{-0.5}$)	0.0274	0.0240–0.0308	$\ln(\lambda) = -2\ln\left(b_5(T-3.37)\{1-\exp[c_5(T-44.7)]\}\right)$ (model 2)
T_{min5} (°C)	3.37	Fixed	
c_5 (°C^{-1})	0.373	0.228–0.518	
T_{max5} (°C)	44.7	Fixed	
b_5 (°C^{-1} h$^{-0.5}$)	0.0299	0.0268–0.0329	$\ln(\lambda) = -2\ln\left(b_5(T-3.37)\{1-\exp[0.256(T-44.7)]\}\right)$ (model 3)
T_{min5} (°C)	3.37	Fixed	
c_5 (°C^{-1})	0.256	Fixed	
T_{max5} (°C)	44.7	Fixed	

[a] See Table 1, footnote c.

Additionally, the A, μ, and λ data are transformed (square root, logarithmic, and reciprocal root transformations) and the variances of the transformed data are calculated (alternative procedure). A linear regression is also performed with these data. These regression data with Student's t test values are also given in Table 2.

Figure 1 shows that the variance of the asymptote (A) shows no clear correlation with the mean. This also holds for the variance divided by the mean and divided by the square of the mean. This is confirmed in Table 2, where it can be seen that with both methods (Ratkowsky [3] and an alternative procedure), no significant correlation is found for all cases. Therefore, it can be concluded that no transformation should be used for the asymptote data.

In Fig. 2, it can be seen that the variance of the growth rate (μ) shows a positive correlation with the mean. The variance divided by the mean shows no clear correlation, and the variance divided by the square of the mean shows a negative correlation. In Table 2, it can be seen that the variance of the growth rate is indeed significantly correlated with the mean. The variance divided by the mean, divided by the square of the mean, and divided by the cube of the mean shows no significant correlation. If the growth rate data are transformed (alternative procedure), all cases give a significant correlation except for the square root transformation. Therefore, the square root transformation was chosen to stabilize the variance of the growth rate data.

In Fig. 3, it can be seen that the variance of the lag time (λ) divided by the mean shows a positive correlation with the logarithm of the mean (because of the large range of lag time values, a logarithmic transformation is used in these graphs). The variance divided by the square of the mean shows no correlation, and the variance divided by cube of the mean shows a negative correlation. In Table 2, it can be seen that the variance and the variance divided by the mean shows a significant correlation. If the lag time data are transformed (alternative procedure), no transformation and a square root transformation give a significant correlation. The logarithm and reciprocal root transformation give no significant correlation. Therefore, the logarithmic transformation was chosen to stabilize the variance of the lag time data.

TABLE 6. Statistical tests for the ln (λ) models[a]

Model no.	No. of parameters	DF	RSS	MS	f	F
Model 1	2	36	9.70	0.269		
LOF 1		16	2.00	0.125	0.325	2.2
Model 2	2	36	12.65	0.351		
LOF 2		16	4.95	0.309	0.803	2.2
Model 3	1	37	14.03	0.379		
LOF 3		17	6.33	0.372	0.967	2.2
General	18	20	7.70	0.385		

[a] See Table 4, footnote a.

Model update. Now that the correct stabilizing transformations for A, μ, and λ have been found, the parameter values of the previously proposed models (6) can be updated. For the asymptote (A), no transformation was used in our former model development, and now it has been shown that transformation is not necessary. Therefore, the model and model parameters determined from the original data set remain unchanged (Table 1). The square root transformation is the best transformation to stabilize the variance of the growth rate data (μ). Therefore, the model should be fitted to the square root of the data. The results of fitting the previously measured data (6) to the square root relation are given in Table 3. The lack of fit of the model is compared with the measurement error, and the square root relation was accepted on basis of the F test (Table 4).

For the lag time data (λ), the logarithmic transformation stabilizes the variance and was already used (Table 1).

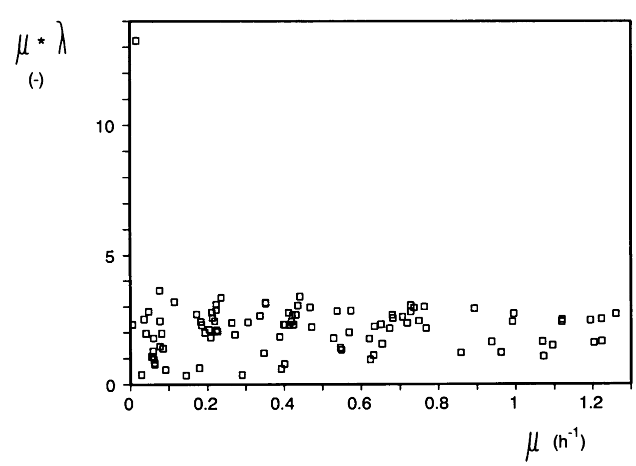

FIG. 4. μ multiplied by λ plotted against μ.

TABLE 7. Parameter values and models for the effect of temperature on the asymptote (A), growth rate (μ_m), and lag time (λ) for *L. plantarum* in MRS medium based on original data set (6)

Parameter	Model and equation	Variable	Value
A	Ratkowsky 4, $A = b_4\{1 - \exp[c_4(T - T_{max4})]\}$	b_4 (−)	8.46
		c_4 (°C^{-1})	1.25
		T_{max4} (°C)	43.1
μ_m	Ratkowsky, $\sqrt{\mu_m} = b_2(T - T_{min2})\ \{1 - \exp[c_2(T - T_{max2})]\}$	b_2 (°C^{-1} h$^{-0.5}$)	0.0385
		T_{min2} (°C)	3.37
		c_2 (°C^{-1})	0.256
		T_{max2} (°C)	44.7
λ	*Reciprocal atkowsky, n* (λ) $= -\ln\left(b_5(T - T_{min5})\ \{1 - \exp[c_5(T - T_{max5})]\}\right)$	b_5 (°C^{-1} h$^{-0.5}$)	0.0299
		T_{min5} (°C)	3.37
		c_5 (°C^{-1})	0.256
		T_{max5} (°C)	44.7

However, the previously proposed reciprocal of the Ratkowsky model (equation 6) was also tested. If it is assumed that the T_{min5} and T_{max5} values are equal to the T_{min2} and T_{max2} values of the equation describing the growth rate (equation 5), this model also contains two parameters. If the c_5 value is also fixed, the model contains only one parameter. The results of fitting the previously measured lag time data to the hyperbolic model and reciprocal square root relation are given in Table 5. The reciprocal Ratkowsky model with all parameters fixed (equation 10) except for b is accepted by the F test (Table 6):

$$\mu = \left(b_2(T - T_{min2}) \cdot \{1 - \exp[c_2(T - T_{max2})]\}\right)^2 \quad (5)$$

$$\lambda = \left(b_5(T - T_{min2}) \cdot \{1 - \exp[c_2(T - T_{max2})]\}\right)^{-2} = \left(\frac{b_2}{b_5}\right)^2 \cdot \frac{1}{\mu} \quad (10)$$

This result indicates that the lag time is reciprocally proportional to the growth rate. This has been suggested by Simpson et al. (4). Similar results for the T_{min} value for the growth rate and lag time are also given by Chandler and McMeekin (2) and Smith (5). The multiplication of the growth rate and lag time is given in Fig. 4. This graph shows that the growth rate and the lag time are reciprocally proportional (except for one point) over a large range of growth rate values. This model now contains only one parameter and predicts a lag time increase at higher temperature.

The updated models and parameter values, based on the previously measured data (6), are given in Table 7.

Model validation. Now the updated model (based on the earlier 38 experiments) can be used to predict the newly measured data (60 growth curves measured at 17 different temperatures). The newly measured growth parameters are plotted with the predictions in Fig. 5, 6, and 7. The growth rate is transformed with a square root, and the lag time is transformed with a logarithmic transformation. From these graphs, it can be concluded that the curves predicted from the parameter estimates obtained for the earlier data set (6) are not inconsistent with the A, μ, and λ values that were obtained from the new (independent) data set. For the

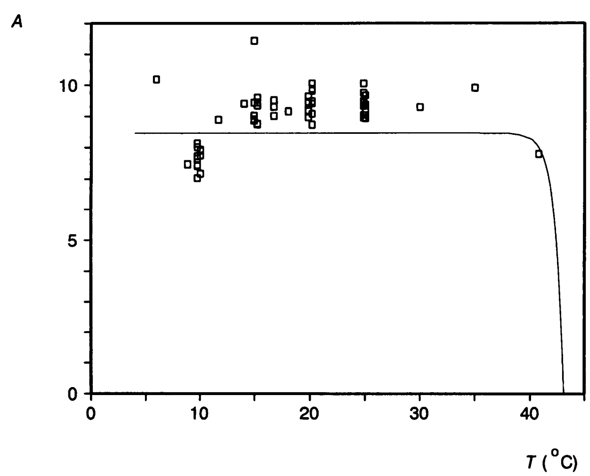

FIG. 5. New asymptote data and model predictions (——) based on previous data.

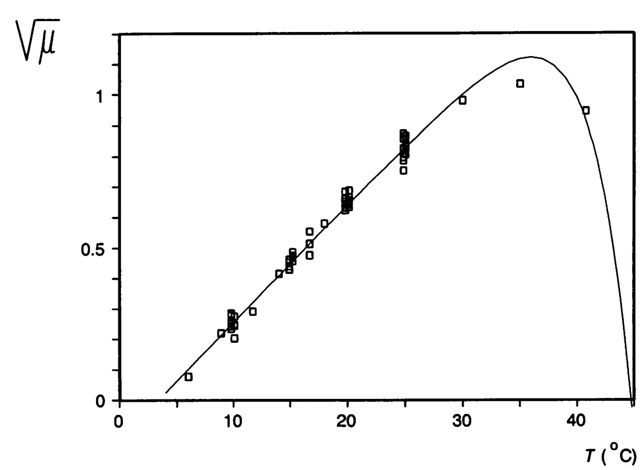

FIG. 6. New growth rate data and model predictions (——) based on previous data.

TABLE 8. Results of the F test comparing new data and model[a]

Test	A	$\sqrt{\mu_m}$	ln (λ)	
RSS_{error}	31.3	0.125	14.0	
DF_{error}	35	34	37	
MS_{error}	0.895	0.00367	0.379	
	Ratkowsky 4	Ratkowsky	Hyperbola	$(Ratk)^{-1}$
RSS_g (DF_g = 43)	9.79	0.0269	2.39	2.39
RSS_m (DF_m = 60)	59.0	0.0425	13.1	10.4
LOF (DF_{LOF} = 17)	49.2	0.0157	10.8	8.00
MS_{LOF}	2.90	0.000921	0.633	0.470
f	3.24	0.251	1.67	1.24

[a] RSS, residual sum of squares; DF, degrees of freedom; MS, mean square; error, error of previous data; RSS_g, RSS of general model (mean of replicates); RSS_m, RSS of model prediction; $(Ratk)^{-1}$ reciprocal Ratkowsky model (equation 6); LOF, lack of fit ($RSS_m - RSS_g$); f, MS_{LOF}/MS_{error}. F table value (95% confidence), $F_{34}^{17} \approx 2.00$.

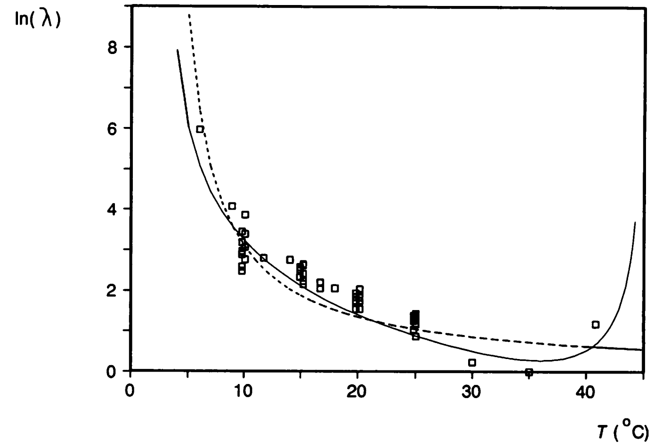

FIG. 7. New lag time data and reciprocal Ratkowsky model (——) and hyperbolic model (- - -) predictions based on previous data.

growth rate data, the residuals show no trend. However, the lag time data and the asymptote data show some discrepancies (the residuals are not randomly distributed around zero). It should be noted that the new data were all obtained at temperatures below 40°C. Therefore, the models are only validated within the range from 6 to 40°C. Furthermore, it should be noted that the new data in Fig. 5, 6, and 7 are compared with predictions obtained by using parameter values determined with other data (no fitting).

The lack of fit of the models is compared with the measurement error by the F test (Table 8). The mean square of the lack of fit must be tested against the mean square of the measurement error. The measurement error is estimated by calculating the deviation of the first set of data with the growth model (6). The lack of fit of the new data is calculated by subtracting the RSS of the growth-temperature model (RSS_m with $DF_m = 60 - 0 = 60$ [number of observations minus number of estimated parameters]) and the RSS due to the residual variance (RSS_g with $DF_g = 60 - 17 = 43$ [total number of observations minus number of different temperatures measured]).

From this statistical test, we can conclude that for the growth rate data and the lag time data, the deviation between the model prediction and the data is of the same order as the measurement error. The reciprocal Ratkowsky model had a better predictive ability (in this case) than the hyperbola model.

For the asymptote data, however, there is a significant deviation between the model and the data (this can also be seen globally in Fig. 5).

Parameter update. Now that the model has been tested, the parameters can be updated by using all the data together. The parameter values were updated by using all 98 growth curves. The final parameter values are given in Table 9. By comparing the parameters in Table 9 (parameters based on 98 growth curves) and Table 7 (parameters based on 38 growth curves), it can be seen that the update resulted in only small changes. The results are shown graphically in Fig. 8 to 10.

Asymptote model. It is shown that there is a systematic deviation between the model and the asymptote data (Fig. 5 and 8 and Table 8). Therefore, another model was attempted. The following model is proposed:

$$A = a \cdot \frac{(T - T_{min6})(T - T_{max6})}{(T - b_6)(T - c_6)} \quad (11)$$

TABLE 9. Parameter values and models for the effect of temperature on the asymptote (A), growth rate (μ_m), and lag time (λ) for *L. plantarum* in MRS medium, final parameter values based on full data set

Parameter	Model and equation	Variable	Estimate	95% Confidence interval
A	Ratkowsky 4, $A = b_4\{1 - \exp[c_4(T - T_{max4})]\}$	b_4 (–)	8.83	8.63–9.02
		c_4 (°C^{-1})	1.05	0.679–1.43
		T_{max4} (°C)	43.2	42.9–43.4
μ_m	Ratkowsky, $\sqrt{\mu_m} = b_2(T - T_{min2})\{1 - \exp[c_2(T - T_{max2})]\}$	b_2 (°C^{-1} h$^{-0.5}$)	0.0385	0.0368–0.0402
		T_{min2} (°C)	3.29	2.63–3.95
		c_2 (°C^{-1})	0.247	0.207–0.288
		T_{max2} (°C)	44.8	44.4–45.2
λ	Reciprocal Ratkowsky, ln (λ) =	b_5 (°C^{-1} h$^{-0.5}$)	0.0276	0.0263–0.0289
	$-2\ln\left(b_5(T - T_{min5})\{1 - \exp[c_5(T - T_{max5})]\}\right)$	T_{min5} (°C)	3.29	Fixed
		c_5 (°C^{-1})	0.247	Fixed
		T_{max5} (°C)	44.8	Fixed

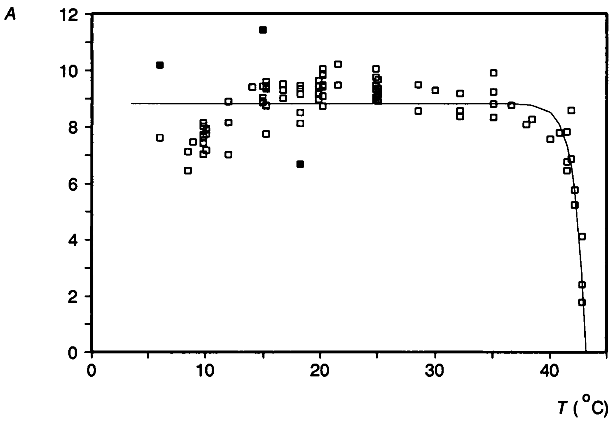

FIG. 8. All asymptote data and updated model fit (——). Solid squares are outlyers.

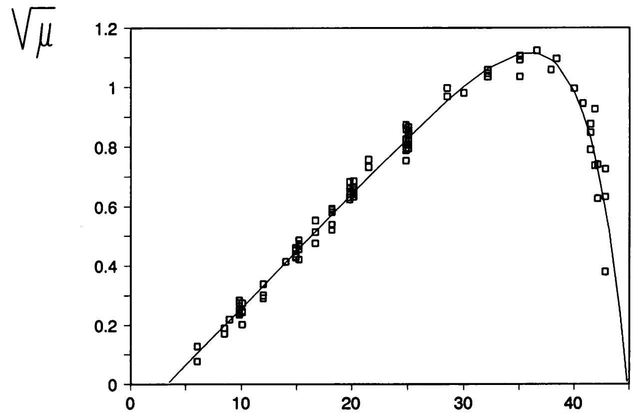

FIG. 9. All growth rate data and updated model fit (——).

In this model, parameter b_6 must be somewhat lower than T_{min6} and c_6 must be a little higher than T_{max6} (b_6 and c_6 being the temperatures at which the asymptote will reach minus infinity). The results of fitting this equation are given in Table 10. The datum points indicated with a solid box in Fig. 8 are not taken into account, since these data deviate widely (outside the 99% prediction interval). The rejection of three points out of 98 seems justified.

It can be seen in Table 11 that the Ratkowsky 4 model is rejected on the basis of the F test and the newly proposed model is accepted. The confidence interval of T_{min6} includes the T_{min2} value of the growth rate data (Tables 9 and 10), so this value can be fixed. The confidence interval of T_{max6} does not include the T_{max2} value of the growth rate data. With a fixed T_{min6} value, the model is also accepted by the F test, and this model is shown graphically in Fig. 11. It shows a decrease in the asymptote values (number of cells ultimately produced) at extremes of temperatures. These effects may result from a relative increase in the maintenance energy at low growth rates. If more energy is consumed for maintenance, a lower cell number will be reached. The decline at low temperatures was mentioned in our previous article (6) but could not be proven statistically with 38 observations. With the current 95 observations, this effect is shown to be statistically significant.

Conclusions. It has been shown with 80 growth curves (curves at suboptimum temperatures) for *L. plantarum* at 17 different temperatures that the asymptote can best be modeled without transformation, the growth rate with a square root transformation, and the lag time with a logarithmic transformation. The choice of the transformation is of eminent importance for the regression analysis of the data.

The previously proposed lag time model has the complication that the lag time at higher temperatures approaches an arbitrary value of 1, whereas at higher temperatures, it can be assumed that the lag phase increases. Therefore, the previously proposed reciprocal of the Ratkowsky model (equation 6) seems better. For this reason, the hyperbolic model is replaced by the reciprocal Ratkowsky model.

The models are validated with new data (60 growth curves at 17 different temperatures). The growth rate data are very well predicted. The reciprocal Ratkowsky model appears to be somewhat better than the hyperbolic model for prediction of the lag phase duration and has the desired ability to increase at higher temperatures. The asymptote data are reasonably well predicted by the Ratkowsky 4 model, but at

TABLE 10. Parameter estimates of the asymptote models

Parameter	Estimate	95% Confidence interval	Model
b_4 (−)	8.80	8.62–8.99	$b_4\{1 - \exp[c_4(T - T_{max4})]\}$ (model 1, Ratkowsky 4)
c_4 (°C^{-1})	1.06	0.711–1.41	
T_{max4} (°C)	43.2	42.9–43.4	
a (−)	10.8	9.83–11.7	$a \cdot \frac{(T - T_{min6})(T - T_{max6})}{(T - b_6)(T - c_6)}$ (model 2)
T_{min6} (°C)	2.20	−1.51–5.92	
T_{max6} (°C)	43.1	42.9–43.2	
b_6 (°C)	−0.352	−6.11–5.41	
c_6 (°C)	43.7	43.4–44.1	
a (−)	10.5	10.1–11.0	$a \cdot \frac{(T - 3.29)(T - T_{max6})}{(T - b_6)(T - c_6)}$ (model 3)
T_{min6} (°C)	3.29	Fixed	
T_{max6} (°C)	43.1	42.9–43.2	
b_6 (°C)	1.29	0.770–1.82	
c_6 (°C)	43.7	43.4–44.0	

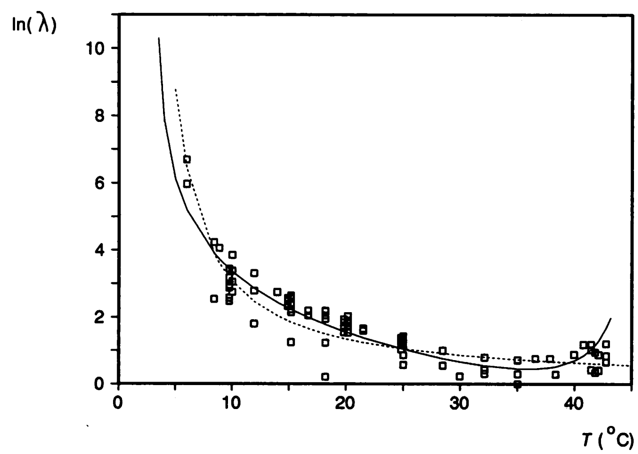

FIG. 10. All lag time data and updated reciprocal Ratkowsky model (——) and hyperbolic model (- - -) fit.

low temperatures, there is a systematic deviation. Therefore, another model (equation 11) is proposed which describes the behavior at low temperatures much better. The decline at low temperatures can now be proven statistically with the current 95 observations. For kinetic predictions, the lag time and the growth rate are the most important parameters.

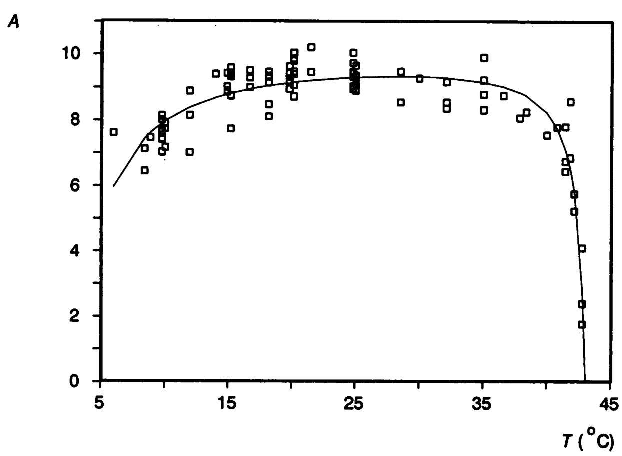

FIG. 11. All asymptote data and new model fit (——).

TABLE 11. Statistical test of the asymptote models[a]

Model no.	No. of parameters	DF	RSS	MS	f	F
Model 1	3	92	65.4	0.711		
LOF 1		26	46.1	1.77	6.08	
Model 2	5	90	32.3	0.359		
LOF 2		24	13.0	0.543	1.86	2.0
Model 3	4	91	32.6	0.359		
LOF 3		25	13.4	0.536	1.84	
General	29	66	19.2	0.292		

[a] See Table 4, footnote a.

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