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Modeling of Bacterial Growth with Shifts in Temperature

M. H. ZWIETERING,[1]* J. C. DE WIT,[1] H. G. A. M. CUPPERS,[2] AND K. VAN 'T RIET[1]

Department of Food Science, Agricultural University Wageningen, 6700 EV Wageningen,[1] and Unilever Research Laboratorium, Vlaardingen,[2] The Netherlands

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The temperature of chilled foods is an important variable for the shelf life of a product in a production and distribution chain. To predict the number of organisms as a function of temperature and time, it is essential to model the growth as a function of temperature. The temperature is often not constant in various stages of distribution. The objective of this research was to determine the effect of shifts in temperature. The suitability and usefulness of several models to describe the growth of *Lactobacillus plantarum* with fluctuating temperatures was evaluated. It can be assumed that temperature shifts within the lag phase can be handled by adding relative parts of the lag time to be completed and that temperature shifts within the exponential phase result in no lag phase. With these assumptions, the kinetic behavior of temperature shift experiments was reasonably well predicted, and this hypothesis was accepted statistically in 73% of the cases. Only shifts of temperature around the minimum temperature for growth showed very large deviations from the model prediction. The best results were obtained with the assumption that a temperature shift (within the lag phase as well as within the exponential phase) results in an additional lag phase. This hypothesis was accepted statistically in 93% of the cases. The length of the additional lag phase is one-fourth of the lag time normally found at the temperature after the shift.

Temperature is a major factor determining the kinetics of food deterioration reactions. As microbial spoilage is of major concern, the effect of temperature on the specific growth rate of microorganisms is important. Various models to describe the effect of a constant temperature have been described by Zwietering et al. (6, 7). However, the temperature is often not constant during distribution. It can be assumed, for instance, that microorganisms respond instantaneously to changes in temperature. Another possibility is that organisms need to adapt to the new temperature, so that they go through a lag phase caused by the stress of the temperature shift.

Bacteria often show a lag phase after inoculation. The duration of this lag phase is dependent on temperature. If the temperature is changed during incubation, an additional lag may occur. Simpson et al. (5) proposed to calculate the lag time during changing temperature conditions by adding the relative parts of the lag phase. If a culture has completed half of the lag time at the first temperature of incubation and is then transferred to a new temperature, it still has to complete half of the lag time at the new temperature. Langeveld and Cuperus (2) found that bacteria within the exponential phase respond immediately to a change in temperature. Ng et al. (3) and Shaw (4) also carried out experiments with temperature shifts during the exponential phase, and they found that shifts in the moderate temperature range resulted in immediate exponential growth at the growth rate associated with the new temperature. However, they found that shifts to or from low temperatures resulted in an adaptation period. Fu et al. (1) found a significant effect of a temperature step in both the exponential and the lag phase. Biologically, this can be expected, since the cells are out of balance and need to adjust, for instance, their enzyme pool, to a new equilibrium.

It can be concluded that the literature gives no consistent procedure to model temperature shifts. The objective of this research was to determine the effect of shifts in temperature for bacteria that are still within the lag phase and for bacteria that are growing exponentially. Several models are proposed to make kinetic predictions for bacterial growth with a change in temperature. With a large data set and a statistical analysis, the procedures to handle temperature changes are compared. The suitability and usefulness of these models will be discussed.

THEORY

Description of experimental bacterial growth data. Growth curves are defined as the logarithm of the relative population size [$y = \ln (N/N_0)$] as a function of time (t). A growth model with three parameters can describe the growth curve (8). One method to describe a growth curve is to assume no growth within the lag phase or within the asymptotic phase and to assume exponential growth within the exponential phase:

$$\begin{aligned} y &= 0 && t \leq \lambda \\ y &= \mu \cdot (t - \lambda) && \lambda < t < A/\mu + \lambda \\ y &= A && t \geq A/\mu + \lambda \end{aligned} \tag{1}$$

Since bacterial growth curves often show a sigmoidal shape, a second method is to describe the data by a sigmoidal function, for example, the modified Gompertz equation (8). The specific growth rate (μ_m), the lag time (λ), and the asymptote (A) can be determined from growth data by fitting them to this modified Gompertz model:

* Corresponding author. Mailing address: Department of Food Science, Agricultural University Wageningen, P.O. Box 8129, 6700 EV Wageningen, The Netherlands. Phone: 31-8370-84204. Fax: 31-8370-82237. E-mail: MARCEL.ZWIETERING@PROCK.LMT.WAU.NL.

TABLE 1. Parameter values and models for the effect of temperature on the asymptote (A), specific growth rate (μ), and lag time (λ) for *L. plantarum* in MRS medium (6)

Parameter	Model and equation	Variable[a]	Value
A	$A = a\frac{(T - T_{min6})(T - T_{max6})}{(T - b_6)(T - c_6)}$	a (−)	10.5
		T_{min6} (°C)	3.29
		T_{max6} (°C)	43.1
		b_6 (°C)	1.29
		c_6 (°C)	43.7
μ	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)	3.29
		c_2 (°C^{-1})	0.247
		T_{max2} (°C)	44.8
λ	Reciprocal Ratkowsky, $\ln (\lambda) = -2\ln \left(b_5(T - T_{min5}) \{1 - \exp [c_5(T - T_{max5})]\} \right)$	b_5 (°C^{-1} h$^{-0.5}$)	0.0276
		T_{min5} (°C)	3.29
		c_5 (°C^{-1})	0.247
		T_{max5} (°C)	44.8

[a] For parameter a, 23.62 − ln (N_0) must be used if different inoculum amount is used.

$$y = A \exp \left\{ - \exp \left[\frac{\mu_m \cdot e}{A} (\lambda - t) + 1 \right] \right\} \quad (2)$$

Growth-temperature relations. Models to describe the effect of a constant temperature on the asymptote (A), specific growth rate (μ), and lag time (λ), with parameter values, are given in Table 1 (6).

Growth with temperature changes. Cultures are shifted at time t_s from temperature T_1 to T_2. Growth parameters (at a constant temperature, determined from Table 1) have index 1 before the temperature shift and index 2 after the shift [$A(T_1) = A_1; A(T_2) = A_2; \mu(T_1) = \mu_1; \mu(T_2) = \mu_2; \lambda(T_1) = \lambda_1; \lambda(T_2) = \lambda_2$]. The dimensionless time of shift (t_s^*) is defined as t_s divided by λ_1. From the growth data after the shift, a lag time, λ_{shift}, can be estimated. Several hypotheses can be proposed to predict this λ_{shift}.

Temperature shift within the lag phase ($t_s \leq \lambda_1$, $t_s^* \leq 1$). If bacteria are subjected to a temperature shift from T_1 to T_2 during the lag phase, they will not have completed their lag period and will still show a lag phase at the new temperature. Three hypotheses are being tested.

(i) The effect of the temperature shift results in a new lag phase that is equal to the lag phase normally found at T_2:

$$\lambda_{shift} = \lambda_2 \quad (3)$$

In this case, it is assumed that the incubation at T_1 had no effect and that the shift disturbs the cells in such a manner that they start the full lag period over again.

TABLE 2. Relative lag time (λ^*) resulting from the three hypotheses

Hypothesis	Shift within lag phase ($t_s^* \leq 1$)	Shift within exponential phase ($t_s^* > 1$)
1	1	1
2	$1 - t_s^*$	0
3	$1 + \gamma - t_s^*$	γ

(ii) The effect of the temperature shift results in a new lag phase that is equal to the relative part of the lag phase that still has to be completed. If, for instance, one-third of the lag phase is completed during incubation at the first temperature ($t_s = \frac{1}{3}\lambda_1$; $t_s^* = \frac{1}{3}$), two-thirds of the lag time still has to be completed at the temperature after the shift ($\lambda_{shift} = \frac{2}{3}\lambda_2$). For a general case, this can be written as:

$$\lambda_{shift} = \left(1 - \frac{t_s}{\lambda_1}\right) \cdot \lambda_2 = (1 - t_s^*) \cdot \lambda_2 \quad (4)$$

(iii) It can also be expected that a temperature shift results in an additional lag phase since the cells are stressed by the temperature shift. Therefore, it is assumed that a temperature shift results in a new lag phase that is equal to the relative part of the lag phase that still has to be completed plus an additional lag due to the shift in temperature:

$$\lambda_{shift} = (1 - t_s^*) \cdot \lambda_2 + \lambda_x \quad (5)$$

where λ_x is the additional lag due to the shift in temperature.

If we now assume that the additional lag due to the shift in

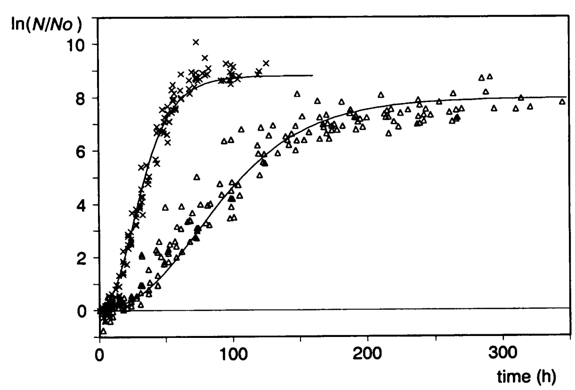

FIG. 1. Growth curves of *L. plantarum* at 10 (△) and 15°C (×) compared with model predictions (——) at constant temperature.

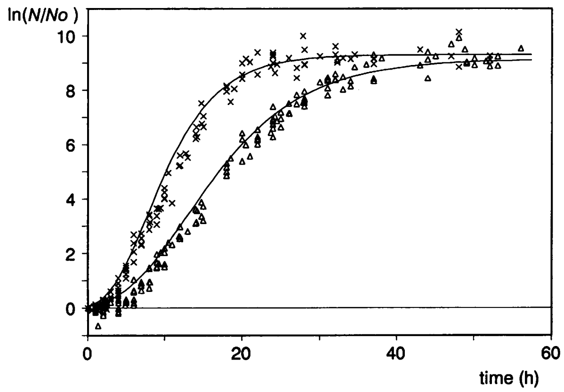

FIG. 2. Growth curves of *L. plantarum* at 20 (△) and 25°C (×) compared with model predictions (——) at constant temperature.

temperature is proportional to the lag phase at T_2 ($\lambda_x = \gamma \cdot \lambda_2$), we get:

$$\lambda_{shift} = (1 + \gamma - t_s^*) \cdot \lambda_2 \qquad (6)$$

where γ is the proportionality constant.

Temperature shift within the exponential phase ($t_s > \lambda_1$, $t_s^* > 1$). Again, three hypotheses are being tested.

(i) The effect of the temperature shift gives such a disturbance that it results in a new lag phase that is equal to the lag phase normally found at T_2:

$$\lambda_{shift} = \lambda_2 \qquad (7)$$

(ii) If it is assumed that the bacteria show no surplus lag phase due to a change in temperature during the exponential phase, growth continues immediately at the specific growth rate associated with T_2:

$$\lambda_{shift} = 0 \qquad (8)$$

(iii) It can also be assumed that a temperature shift results in an additional lag phase (analogous to equations 5 and 6) since the cells are stressed by the temperature shift:

$$\lambda_{shift} = \lambda_x = \gamma \cdot \lambda_2 \qquad (9)$$

where λ_x is the additional lag due to the shift in temperature and γ is the proportionality constant.

Summary of hypotheses. In all the above-mentioned hypotheses, the lag time after the shift (λ_{shift}) is proportional to λ_2. Therefore, a relative lag time (λ^*) can be defined as:

$$\lambda^* = \frac{\lambda_{shift}}{\lambda_2} \qquad (10)$$

A summary of the effect of shifts within the lag and exponential phases for the three hypotheses on this λ^* value is given in Table 2. Note that hypothesis 3 is equal to hypothesis 2 for $\gamma = 0$.

MATERIALS AND METHODS

Microbial experiments. The culture of *Lactobacillus plantarum* (American Type Culture Collection [ATCC] determined; no ATCC number) was stored frozen at −16°C. The bacteria were cultivated twice at 30°C, first for 24 h and then for 16 h. The amount of this culture inoculated into test tubes for individual growth trials was 0.01% (about 5 × 10^5 organisms) and in some cases 0.0001% (about 5 × 10^3 organisms). All incubations were performed in MRS medium (Difco Laboratories) in a temperature gradient incubator (7). Growth was measured by plate counts on pour plates (MRS medium with 12 g of agar [Agar Technical, Oxoid Ltd.] per liter).

Ten independent growth curves at constant temperature were measured at 10, 15, 20, and 25°C. The combined datum points for each temperature were compared with model predictions, and the resulting residual sum of squares (RSS) was used to estimate the experimental measurement error.

Temperature shifts within the lag phase were carried out in 71 experiments. Temperature shifts within the exponential phase were carried out in 53 experiments.

Temperature shifts. Temperature shifts were carried out by moving tubes from one temperature to another temperature in the gradient incubator. Tubes had an inner diameter of 1.35 cm and were filled with 10 ml of medium, resulting in a liquid height of 7 cm. Temperature responses were measured at different temperature steps. After 8 min, the temperature difference between the tube and the incubator was less than 10% of the step value; after 16 min, this value was less than 1%.

Comparison of hypotheses. The variance of the measurement error at different temperatures (for constant temperature experiments) was determined by calculating the RSS of the data at these temperatures (10, 15, 20, and 25°C,

TABLE 3. RSS of the combined datum points for different temperatures described by model predictions from previously validated models (6)

Temp (°C)	RSS	No. of datum points (n)	Variance (RSS/n)
10	71.3	191	0.374
15	25.4	151	0.168
20	22.6	170	0.133
25	28.1	134	0.210
Pooled	147.5	646	0.2283 (= var_{me})

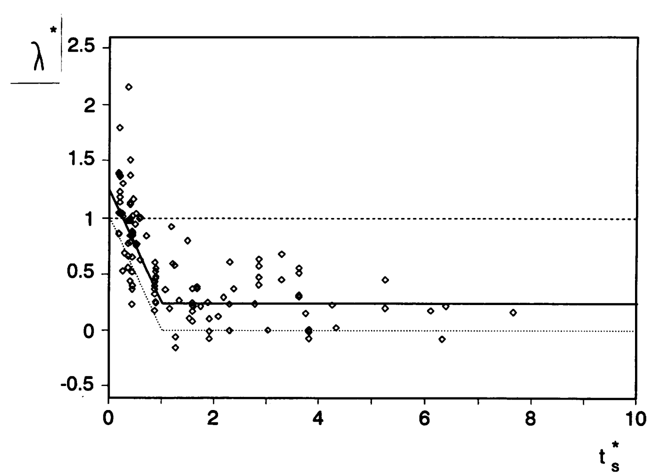

FIG. 3. Relative lag time (◇) plotted versus the relative time of shift compared with model predictions with hypothesis 1 (– – –), hypothesis 2 (· · · ·), and hypothesis 3 with γ = 0.25 (——).

TABLE 4. Results of temperature shifts within the lag phase[a]

Curve no.[b]	Temp (°C)		t_s (h)	n	λ_1 (h)	λ_2[c] (h)	t_s^*	λ (h)	95% Confidence limits[d]		Lag time predicted[e]	
	T_1	T_2							Minimum	Maximum	$\gamma = 0$	$\gamma = 0.25$
L1	14.9	10.1	2.50	17	9.76	28.36	0.256	15.11	4.31	25.91	**21.10**	28.19
L2	25.1	10.1	1.25	15	2.81	28.36	0.445	11.43	−0.60	23.45	**15.73**	**22.82**
L3	24.8	10.0	1.25	18	2.88	29.21	0.434	6.90	−4.57	18.37	**16.54**	23.84
L4	25.0	9.9	1.25	18	2.83	30.10	0.442	11.09	−0.24	22.43	**16.81**	24.34
L5	24.8	9.7	1.25	21	2.88	32.01	0.434	16.77	6.85	26.69	**18.12**	**26.13**
L6	14.9	10.1	5.00	17	9.76	**28.36**	0.512	21.52	9.88	33.15	**13.84**	**20.93**
L7	25.1	10.1	2.50	15	2.81	28.36	0.891	6.95	−2.99	16.89	**3.09**	**10.18**
L8	24.8	10.0	2.50	18	2.88	29.21	0.868	5.28	−5.32	15.88	**3.87**	**11.17**
L9	25.0	9.9	2.50	18	2.83	30.10	0.883	7.79	−2.52	18.11	**3.52**	**11.04**
L10	24.8	9.7	2.50	21	2.88	32.01	0.868	12.83	3.94	21.71	**4.24**	**12.24**
L11	10.5	15.3	10.0	13	25.30	**9.13**	0.395	8.92	5.00	12.85	**5.52**	**7.80**
L12	9.9	15.1	6.00	12	30.10	**9.44**	0.199	10.80	6.46	15.15	**7.56**	**9.92**
L13	9.9	15.1	6.00	14	30.10	**9.44**	0.199	11.23	7.39	15.06	**7.56**	**9.92**
L14	9.9	15.0	6.00	16	30.10	**9.60**	0.199	11.43	7.65	15.20	**7.69**	**10.09**
L15	9.6	14.9	6.00	17	33.03	**9.76**	0.182	8.45	4.57	12.34	**7.99**	**10.43**
L16	25.1	15.2	1.25	12	2.81	**9.28**	0.445	8.11	4.26	11.97	**5.15**	**7.47**
L17	24.8	15.1	1.25	14	2.88	**9.44**	0.434	8.01	4.23	11.79	**5.34**	**7.70**
L18	25.0	15.0	1.25	17	2.83	**9.60**	0.442	8.22	4.51	11.93	**5.36**	**7.76**
L19	24.8	15.0	1.25	18	2.88	**9.60**	0.434	6.30	2.62	9.98	**5.43**	**7.83**
L20	10.5	15.3	15.0	12	25.30	**9.13**	0.593	9.22	5.18	13.25	3.72	**6.00**
L21	9.9	15.1	12.0	11	30.10	**9.44**	0.399	7.43	3.01	11.85	**5.68**	**8.03**
L22	9.9	15.1	12.0	14	30.10	**9.44**	0.399	9.45	5.80	13.09	**5.68**	**8.03**
L23	9.6	15.0	12.0	15	33.03	**9.60**	0.363	6.39	2.55	10.24	**6.11**	**8.51**
L24	9.6	14.9	12.0	16	33.03	9.76	0.363	5.50	1.70	9.30	**6.22**	**8.66**
L25	25.1	15.2	2.50	12	2.81	9.28	0.891	4.47	0.69	8.24	**1.01**	**3.33**
L26	24.8	15.1	2.50	12	2.88	9.44	0.868	4.08	0.50	7.66	**1.25**	**3.61**
L27	25.0	15.0	2.50	16	2.83	9.60	0.883	4.47	0.90	8.04	**1.12**	**3.52**
L28	24.8	15.0	2.50	17	2.88	9.60	0.868	3.55	−0.25	7.34	**1.27**	**3.67**
L29	10.5	20.2	10.0	15	25.30	**4.62**	0.395	3.91	2.32	5.51	**2.79**	**3.95**
L30	9.9	19.9	6.00	14	30.10	**4.78**	0.199	5.06	3.25	6.86	**3.83**	**5.03**
L31	9.9	19.9	6.00	14	30.10	**4.78**	0.199	6.56	4.49	8.63	3.83	**5.03**
L32	9.6	20.0	6.00	16	33.03	**4.73**	0.182	6.58	4.57	8.58	3.87	**5.05**
L33	9.6	19.6	6.00	16	33.03	**4.96**	0.182	4.28	2.46	6.09	**4.06**	**5.30**
L34	14.9	20.3	2.50	13	9.76	**4.56**	0.256	4.64	2.33	6.96	**3.40**	**4.54**
L35	25.1	20.3	1.25	14	2.81	**4.56**	0.445	3.87	1.96	5.77	**2.53**	**3.67**
L36	24.8	19.8	1.25	14	2.88	**4.84**	0.434	4.23	2.33	6.14	**2.74**	**3.95**
L37	25.0	19.8	1.25	13	2.83	**4.84**	0.442	4.96	2.94	6.97	2.70	**3.91**
L38	24.8	19.9	1.25	14	2.88	**4.78**	0.434	4.23	2.35	6.11	**2.71**	**3.91**
L39	10.5	20.2	15.0	12	25.30	**4.62**	0.593	4.64	2.57	6.72	1.88	**3.03**
L40	9.9	19.9	12.0	12	30.10	4.78	0.399	2.12	−0.28	4.52	**2.88**	**4.07**
L41	9.9	19.9	12.0	11	30.10	**4.78**	0.399	5.47	2.52	8.42	**2.88**	**4.07**
L42	9.6	20.0	12.0	13	33.03	**4.73**	0.363	4.63	1.86	7.41	**3.01**	**4.19**
L43	9.6	19.6	12.0	13	33.03	**4.96**	0.363	3.87	1.21	6.53	**3.16**	**4.40**
L44	14.9	20.3	5.00	13	9.76	**4.56**	0.512	4.77	2.08	7.46	**2.23**	**3.37**
L45	25.1	20.3	2.50	13	2.81	4.56	0.891	2.54	0.54	4.54	0.50	**1.64**
L46	24.8	19.8	2.50	13	2.88	4.84	0.868	1.59	−0.24	3.41	**0.64**	**1.85**
L47	25.0	19.8	2.50	13	2.83	4.84	0.883	2.56	0.71	4.41	0.57	**1.78**
L48	24.8	19.9	2.50	15	2.88	4.78	0.868	2.11	0.39	3.84	**0.63**	**1.83**
L49	10.5	25.0	10.0	13	25.30	**2.83**	0.395	3.19	1.96	4.42	1.71	**2.42**
L50	9.9	24.9	6.00	11	30.10	**2.86**	0.199	3.54	2.17	4.91	**2.29**	**3.00**
L51	9.9	24.9	6.00	14	30.10	**2.86**	0.199	3.94	2.69	5.18	2.29	**3.00**
L52	9.6	25.0	6.00	12	33.03	**2.83**	0.182	3.98	2.66	5.29	2.32	**3.02**
L53	9.6	24.7	6.00	15	33.03	**2.91**	0.182	3.05	1.84	4.27	**2.38**	**3.11**
L54	14.9	25.1	2.50	12	9.76	**2.81**	0.256	3.68	2.46	4.89	2.09	**2.79**
L55	20.2	25.1	1.33	15	4.62	**2.81**	0.288	1.95	1.01	2.88	**2.00**	**2.70**
L56	10.5	25.0	15.0	13	25.30	**2.83**	0.593	1.79	0.43	3.14	**1.15**	**1.86**
L57	9.9	24.9	12.0	10	30.10	**2.86**	0.399	3.94	1.48	6.40	**1.72**	**2.43**
L58	9.9	24.9	12.0	11	30.10	**2.86**	0.399	4.34	1.49	7.18	**1.72**	**2.43**
L59	9.6	25.0	12.0	12	33.03	**2.83**	0.363	6.11	2.14	10.09	1.80	**2.51**
L60	9.6	24.7	12.0	12	33.03	**2.91**	0.363	2.86	0.95	4.77	**1.85**	**2.58**
L61	14.9	25.1	5.00	10	9.76	**2.81**	0.512	2.18	0.63	3.73	**1.37**	**2.07**
L62	20.2	25.1	2.67	13	4.62	**2.81**	0.578	2.80	1.65	3.95	1.18	**1.89**
L63	10.2	30.7	19.5	11	27.55	**1.86**	0.708	1.57	0.82	2.33	0.54	**1.01**
L64	10.2	30.7	24.0	10	27.55	**1.86**	0.871	1.13	0.27	1.99	0.24	**0.70**
L65	4.0	20.0	1873	22	2616	4.73	0.716	22.56	19.83	25.30	1.34	2.52
L66	4.0	35.0	1873	18	2616	1.57	0.716	15.14	14.11	16.16	0.45	0.84

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TABLE 4—*Continued*

Curve no.[b]	Temp (°C)		t_s (h)	n	λ_1 (h)	λ_2[c] (h)	t_s^*	λ (h)	95% Confidence limits[d]		Lag time predicted[e]	
	T_1	T_2							Minimum	Maximum	$\gamma = 0$	$\gamma = 0.25$
L67	14.0	24.0	5.25	16	11.47	**3.10**	0.458	3.64	1.43	5.85	**1.68**	**2.46**
L68	14.0	24.0	2.25	17	11.47	3.10	0.196	5.58	3.55	7.62	2.49	3.27
L69	14.7	20.0	5.00	16	10.11	**4.73**	0.495	4.48	2.57	6.39	2.39	**3.57**
L70	14.7	20.0	2.50	16	10.11	**4.73**	0.247	4.95	2.94	6.95	**3.56**	**4.74**
L71	20.0	30.0	2.50	8	4.73	**1.94**	0.529	1.50	0.29	2.71	**0.91**	**1.40**

[a] t_s, time of shift; n, number of datum points in growth curve; t_s^*, t_s/λ_1; λ, estimated lag time from growth data.
[b] Curves shown in boldface type appear in one of the graphs shown in Fig. 4 through 9.
[c] Values in boldface type indicate that hypothesis 1 is accepted by Student's t test.
[d] Minimum and maximum 95% confidence limits for lag time; 95% confidence intervals are calculated with a Student's t test value of 1.96 (at infinite degrees of freedom, the variance of the measurement error was calculated with 646 datum points).
[e] For $\gamma = 0$, values in boldface type indicate that hypothesis 2 is accepted by student's t test; for $\gamma = 0.25$, values in boldface type indicate that hypothesis 3 is accepted by student's t test.

10 independent growth curves at each temperature) compared with the model predictions (modified Gompertz function with parameter values from Table 1). The pooled variance (var_{mc}) was calculated by dividing the pooled RSS by the total number of datum points (no parameters estimated).

Growth after the temperature shift was examined by fitting y data to the modified Gompertz equation (equation 2). For the lag time experiments, the y value was used as such because this represents growth after the temperature shift, as there is no significant change in bacterial numbers during incubation at temperature T_1 (still within the lag phase). For experiments in the exponential phase, growth after the temperature shift is given by $y - y_s$, where y_s was taken as the y value at the time of shift. The time after the shift was used as the time (t) value.

The lag time predicted by the various hypotheses was compared with the measured lag time after the temperature shift by using Student's t test. The 95% confidence interval of the lag time was calculated with:

$$\lambda_{\mathrm{min,max}} = \hat{\lambda} \pm t_{\mathrm{stud}}\sqrt{V_{\lambda\lambda} \cdot \mathrm{var}_{\mathrm{me}}} \quad (11)$$

where $\hat{\lambda}$ is the estimated lag time value, t_{stud} is the Student's t value, $V_{\lambda\lambda}$ is the diagonal value corresponding to λ in the Jacobian matrix (results from the Marquardt fitting procedure), and $\mathrm{var}_{\mathrm{me}}$ is the variance of the measurement error.

RESULTS AND DISCUSSION

Constant-temperature experiments. In order to calculate confidence intervals, an estimate of the measuring error is necessary. Ten independent growth curves were measured at 10, 15, 20, and 25°C. The combined datum points for each temperature were compared with model predictions from previously devised and validated models (Fig. 1 and 2, Table 1). Figures 1 and 2 show that the dynamic behavior of bacterial growth can be predicted very well. However, exact predictions cannot be made because of experimental errors. The resulting RSS was used to estimate the experimental error (Table 3).

The variances differ at different temperatures; nevertheless, they were pooled to calculate the variance of the measurement error ($\mathrm{var}_{\mathrm{me}}$).

Temperature shift experiments. From the growth curves after the temperature shift, the lag time (λ_{shift}) was estimated by fitting the data to the modified Gompertz equation. This λ_{shift} value was divided by the lag time normally found at T_2 to get the relative lag time (λ^*). The three hypotheses can be tested by plotting this relative lag time (λ^*) versus t_s^* (Fig. 3, Table 2). Figure 3 shows that the relative lag time decreases if t_s^* goes from 0 to 1 and remains constant at higher t_s^* values. Hypothesis 1 assumes that the relative lag time is equal to 1 in all cases. Figure 3 shows that the data do not support this assumption. Therefore, hypothesis 1 seems not to be appropriate. Furthermore, it can be seen that most of the data are above the line predicted by hypothesis 2 ($\gamma = 0$). This indicates that a temperature shift results in an additional lag phase. It was mentioned above that this can be expected, since the cells will be stressed by the temperature shift.

The γ value was estimated by optimizing the statistical acceptance of hypothesis 3 and was found to be 0.25, indicating that a temperature shift results in a new extra lag time (λ_x) equal to one-fourth of the lag time normally found at the temperature after the shift (T_2).

The three hypotheses were tested quantitatively with Student's t test by calculating the confidence interval for the estimated lag time of the growth data after the shift. For the various model predictions, it was tested whether the predictions lie within this confidence interval. The results of experiments with steps within the lag phase are given in Table 4, and those of the experiments with steps within the exponential phase are given in Table 5.

Table 4 shows that hypothesis 1 and hypothesis 2 are accepted for most of the experiments and hypothesis 3 is accepted for almost all experiments for shifts within the lag phase.

In two cases (curves L65 and L66), there were very large deviations between the confidence interval for the measured lag phase duration and the model predictions. The data for these temperature shift experiments are plotted in Fig. 4. The kinetic behavior appears to be reasonably well predicted except for dying cells during incubation at 4°C. However, if the time axis of the part of the curve after the temperature shift is extended (Fig. 5), it can be seen that the predicted lag time is much less than the actual lag time. In these experiments, the first incubation is done at about the minimal temperature for growth (T_{min}, 3.29°C [Table 1]). Incubation at very low temperatures (around or below T_{min}) may be damaging to the cells, resulting in (slow) death and increased lag times when the cells are transferred to higher temperatures. More work is needed to quantify these effects. These two experiments will not be taken into account for the

TABLE 5. Results of temperature shifts within the exponential phase[a]

Curve no.[b]	Temp (°C)		t_s (h)	n	λ_1 (h)	λ_2[c] (h)	t_s^*	λ (h)	95% Confidence limits[d]		Lag time predicted
	T_1	T_2							Minimum	Maximum	$\gamma = 0.25$[e]
E1	8.9	14.2	119	21	41.79	11.06	2.848	4.51	1.39	7.63	**2.76**
E2	8.9	16.5	119	17	41.79	7.55	2.848	4.82	2.33	7.31	1.89
E3	8.9	17.8	119	17	41.79	6.26	2.848	3.00	0.94	5.06	**1.56**
E4	8.9	20.1	119	17	41.79	4.67	2.848	2.70	1.10	4.30	**1.17**
E5	14.0	24.0	72.5	15	11.47	3.10	6.320	−0.22	**−1.77**	1.33	**0.78**
E6	14.0	24.0	70.0	17	11.47	3.10	6.102	0.56	**−0.92**	2.04	**0.78**
E7	14.0	24.0	43.0	6	11.47	3.10	3.748	0.49	**−1.27**	2.25	**0.78**
E8	14.0	24.0	25.0	11	11.47	3.10	2.179	0.92	**−0.26**	2.10	**0.78**
E9	14.0	24.0	20.0	12	11.47	3.10	1.743	0.67	**−0.53**	1.88	**0.78**
E10	14.0	24.0	17.5	14	11.47	3.10	1.525	0.35	**−0.93**	1.62	**0.78**
E11	14.7	20.0	64.5	15	10.11	4.73	6.380	1.04	**−1.10**	3.18	**1.18**
E12	14.7	20.0	43.0	14	10.11	4.73	4.254	1.09	**−0.85**	3.04	**1.18**
E13	14.7	20.0	24.0	14	10.11	4.73	2.374	1.77	**−0.25**	3.80	**1.18**
E14	14.7	20.0	19.0	16	10.11	4.73	1.879	1.19	**−0.31**	2.69	**1.18**
E15	14.7	20.0	16.0	16	10.11	4.73	1.583	1.17	**−0.28**	2.62	**1.18**
E16	14.7	20.0	13.5	17	10.11	4.73	1.335	1.27	**−0.23**	2.78	**1.18**
E17	16.7	10.4	26.5	16	7.32	26.02	3.618	7.75	**−0.86**	16.36	**6.50**
E18	16.7	12.5	26.5	16	7.32	15.51	3.618	4.93	**−0.85**	10.71	**3.88**
E19	16.7	14.6	26.5	15	7.32	10.29	3.618	5.29	0.82	9.76	**2.57**
E20	16.7	18.8	26.5	15	7.32	**5.48**	3.618	3.07	0.28	5.85	**1.37**
E21	18.0	13.9	20.0	21	6.09	11.69	3.284	7.99	4.34	11.64	2.92
E22	18.0	22.1	20.0	17	6.09	3.74	3.284	1.71	0.14	3.27	**0.94**
E23	18.0	13.9	32.0	16	6.09	11.69	5.254	2.36	**−3.17**	7.89	**2.92**
E24	18.0	22.1	32.0	12	6.09	**3.74**	5.254	1.70	**−1.09**	4.50	**0.94**
E25	40.8	25.0	18.0	10	2.35	2.83	7.653	0.47	**−1.68**	2.63	**0.71**
E26	40.8	25.0	3.50	13	2.35	**2.83**	1.488	2.27	0.74	3.80	0.71
E27	9.0	14.0	50.5	15	40.34	**11.47**	1.252	6.69	**−0.07**	13.46	**2.87**
E28	8.0	20.7	70.0	8	59.29	**4.36**	1.181	4.04	1.78	6.30	1.09
E29	11.7	16.6	22.5	14	18.60	**7.43**	1.210	4.44	0.07	8.80	**1.86**
E30	14.2	17.3	18.5	11	11.06	6.71	1.673	2.52	1.13	3.91	**1.68**
E31	14.2	20.5	18.5	12	11.06	4.46	1.673	1.75	0.39	3.11	**1.11**
E32	14.5	24.8	20.0	12	10.47	2.88	1.910	−0.01	**−1.25**	1.23	**0.72**
E33	14.5	30.3	20.0	11	10.47	1.91	1.910	−0.13	**−1.02**	0.76	**0.48**
E34	14.5	34.8	20.0	10	10.47	1.58	1.910	0.17	**−0.58**	0.92	**0.39**
E35	15.3	30.5	19.0	5	9.13	**1.88**	2.082	0.24	**−1.48**	1.97	**0.47**
E36	15.0	25.0	22.0	9	9.60	2.83	2.292	0.67	**−0.47**	1.81	**0.71**
E37	16.6	11.7	22.5	17	7.43	18.60	3.027	0.15	**−9.97**	10.26	**4.65**
E38	16.8	8.0	20.0	15	7.22	59.29	2.772	14.22	**−9.36**	37.79	**14.82**
E39	20.2	8.0	20.0	15	4.62	59.29	4.331	1.69	**−34.30**	37.68	**14.82**
E40	25.0	14.2	4.50	10	2.83	11.06	1.590	4.15	**−0.73**	9.02	**2.76**
E41	25.0	17.3	4.50	10	2.83	6.71	1.590	1.16	**−2.11**	4.43	**1.68**
E42	25.0	18.9	4.50	10	2.83	5.41	1.590	1.22	**−1.28**	3.72	**1.35**
E43	25.0	9.0	6.50	28	2.83	**40.34**	2.296	24.71	8.05	41.37	**10.08**
E44	25.0	11.0	6.50	26	2.83	22.13	2.296	0.06	**−7.48**	7.61	**5.53**
E45	26.0	36.8	3.00	9	2.60	1.57	1.155	0.31	**−0.36**	0.98	**0.39**
E46	30.7	10.1	2.00	9	1.86	28.36	1.075	10.35	1.04	19.65	**7.09**
E47	30.6	10.5	3.00	8	1.87	25.30	1.603	5.47	**−5.36**	16.30	**6.33**
E48	35.0	20.0	2.00	9	1.57	4.73	1.272	−0.71	**−2.21**	0.79	1.18
E49	35.0	20.0	6.00	6	1.57	4.73	3.817	−0.04	**−1.68**	1.60	**1.18**
E50	35.0	25.0	2.50	10	1.57	2.83	1.590	0.24	**−0.90**	1.38	**0.71**
E51	35.0	25.0	6.00	6	1.57	2.83	3.817	0.04	**−1.06**	1.13	**0.71**
E52	35.0	30.0	2.00	11	1.57	1.94	1.272	−0.11	**−0.74**	0.52	**0.49**
E53	35.0	30.0	6.00	6	1.57	1.94	3.817	−0.13	**−1.12**	0.87	**0.49**

[a] See Table 4, footnote *a*.
[b] Curves shown in boldface type are plotted in the graph in Fig. 8 or 9.
[c] See Table 4, footnote *c*.
[d] For the minimum 95% confidence limit for lag time, values in boldface type indicate that $\gamma = 0$ is within the confidence interval (hypothesis 2); for the maximum 95% confidence limit for lag time, 95% confidence intervals are calculated with a Student's t test value of 1.96 (at infinite degrees of freedom, the variance of the measurement error was calculated with 646 datum points).
[e] Values in boldface indicate that hypothesis 3 is accepted by Student's t test.

following discussion. For no other cases was a systematic effect found resulting from the time of the shift, the first or second incubation temperature, the direction of the shift, or the size of the step.

Table 5 shows that hypothesis 1 is accepted for only a few experiments, hypothesis 2 is accepted for most of the experiments, and hypothesis 3 is accepted for almost all experiments for shifts within the exponential phase.

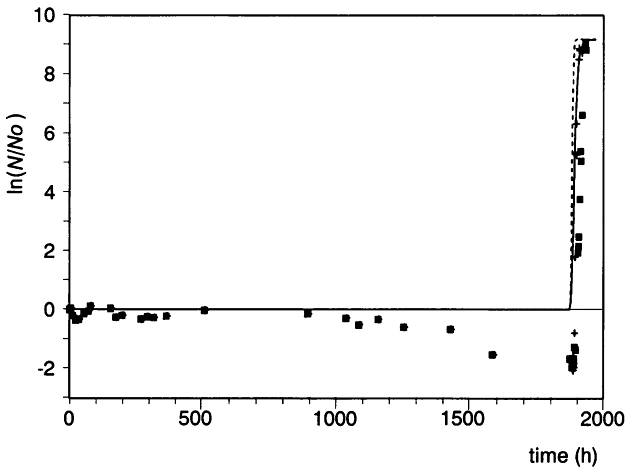

FIG. 4. Growth curve with a shift from 4°C to 20 (L65, ■) or 35°C (L66, +) at 1,873 h compared with model predictions for shift to 20°C (——) and 35°C (- - -).

In Table 6, the total number of accepted cases for each hypothesis is given for all curves (except curves L65 and L66). Table 6 shows that hypothesis 1 is accepted in only 47% of the cases and does not appear to be appropriate (see also Fig. 3). Hypothesis 2 is accepted in most of the cases (73%), and hypothesis 3 is accepted in almost all cases (93%). Therefore, hypothesis 3 appears to be the most appropriate.

Predictions compared with experiments. Now the measured data can be compared with the model predictions. Growth data and model predictions (following hypothesis 3 with $\gamma = 0.25$) are compared for several temperature shift experiments. For shifts within the lag phase, examples are given in Fig. 6 and 7. The model predictions were calculated by using equations A-1 and A-2 (see Appendix). Curves L3 and L4 (Fig. 6) are examples for which hypothesis 3 was rejected.

The results of temperature changes at different times are given in Fig. 8 and 9. For shifts within the exponential phase, the model predictions were calculated with equations A-3 and A-4 (see Appendix). It can be seen that the predictions agree very well with the experimental data. It should be noted that the predictions arise from models based on earlier growth data (6) at constant temperature, so no fitting occurred.

TABLE 6. Acceptance rates for the three hypotheses[a]

Hypothesis	Lag phase (n = 69)	Exponential phase (n = 53)	Total (n = 122)
1	49 (71)	8 (15)	57 (47)
2	51 (74)	38 (72)	89 (73)
3	65 (94)	48 (91)	113 (93)

[a] Values are no. (%) of cases accepted by Student's t test from the total number of cases indicated.

Hypothesis 2 was accepted in many cases (73%), and this assumption is more convenient for simulation purposes, especially if there are many temperature changes during the shelf life of a product or there are dynamically changing temperatures. The predictions made with this assumption ($\gamma = 0$) are also given in Fig. 7. It can be seen that this model also describes the kinetic behavior of the data well enough for a number of practical applications. Curves L51 and L52 are examples for which hypothesis 2 was rejected for temperature shifts within the lag phase.

By using both hypothesis 2 and the linear growth model (equation 1) instead of the modified Gompertz model, the calculations become easier. The predictions of the linear model (with the use of hypothesis 2) are compared with those of the modified Gompertz model (with hypothesis 3) in Fig. 6 and 8. The linear model gives results comparable to those of the modified Gompertz model except that where the growth curve levels off towards the asymptote, the linear model fails to describe the data. The asymptotic level [ln (N/N_0)] is strongly dependent on the inoculum level and often shows large experimental error (6, 7). Moreover, the asymptotic level is often of no real practical importance, since food products are generally spoiled before the asymptotic level is reached. For a number of applications, the

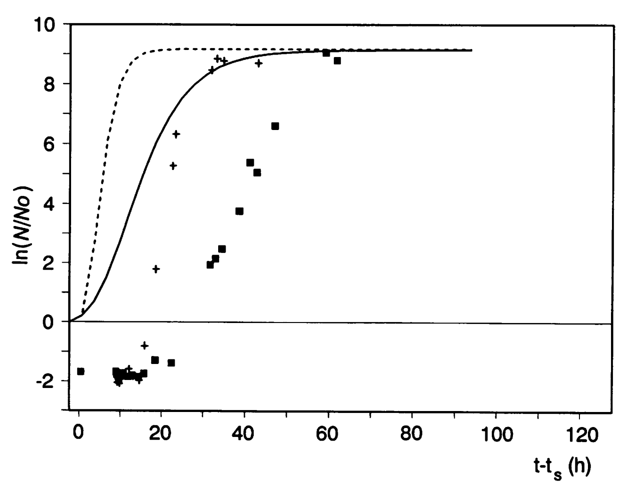

FIG. 5. Growth curve after a shift from 4°C to 20 (L65, ■) or 35°C (L66, +) at 1,873 h compared with model predictions for shift to 20°C (——) and 35°C (- - -). (Magnification of last part of Fig. 4.)

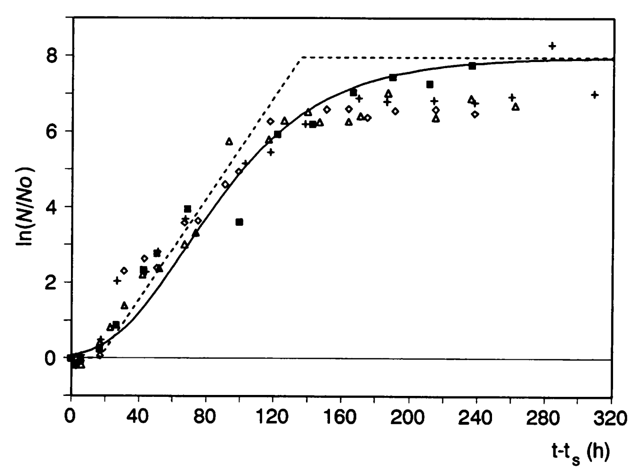

FIG. 6. Growth curves with a shift from 25 to 10°C at 1.25 h compared with predictions with the modified Gompertz model with hypothesis 3 (——) and the linear model with hypothesis 2 (- - -). ■, L2; +, L3; ◇, L4; △, L5).

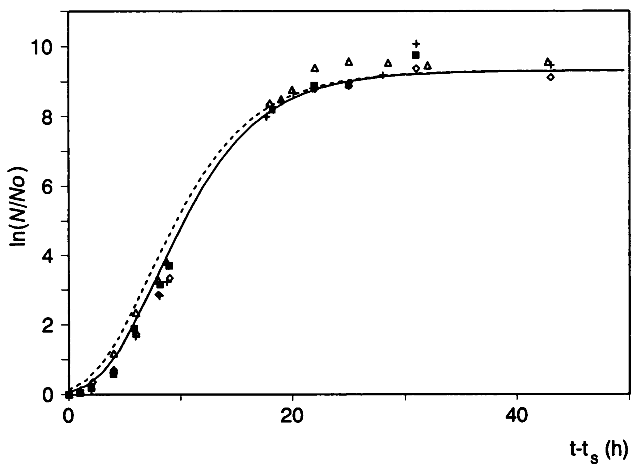

FIG. 7. Growth curves with a shift from 10 to 25°C at 6 h compared with predictions with the modified Gompertz model with hypothesis 3 (——) and with hypothesis 2 (– – –). ■, L50; +, L51; ◇, L52; △, L53).

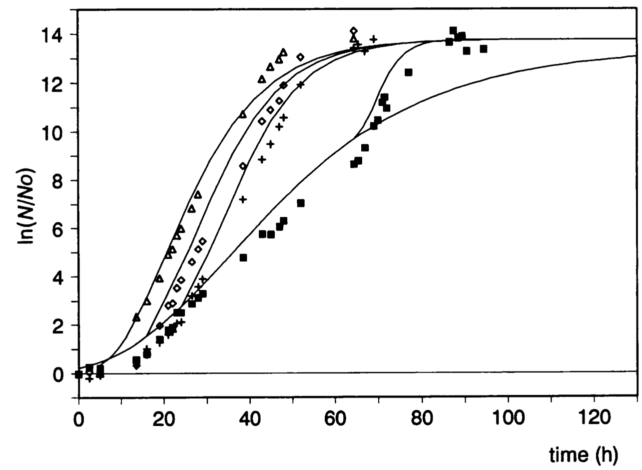

FIG. 9. Growth curves with shifts from 15 to 20°C compared with predictions with the modified Gompertz model with hypothesis 3. ■, E11; +, E13; ◇, E15; △, L69).

global kinetic behavior will be sufficient and the linear model could be chosen, since this model has the advantage of simplicity.

Conclusions. The hypotheses that, for *L. plantarum*, temperature shifts within the lag phase can be handled by adding relative parts of the lag time still to be completed and that temperature shifts within the exponential phase result in no additional lag were accepted statistically in more than 70% of the experiments. The kinetic behavior was well predicted with these assumptions. The hypothesis that a temperature shift results in an additional lag phase of one-fourth of the lag time normally found at the second temperature was accepted in more than 90% of the experiments. This observation shows that the bacteria are exposed to stress by a shift in temperature in the lag phase as well as in the exponential phase.

With this knowledge, growth curves can be predicted with the modified Gompertz equation. For a number of practical applications, the procedure can be simplified by neglecting the additional lag time. The linear growth equation also predicts data within a certain range and therefore can be used when simplicity of the function is preferred.

Shifts of temperature around the minimum temperature of growth showed, first, death of the cells and, second, very large deviations from the model prediction. This observation indicates the need for more experimental work around the minimum temperature of growth.

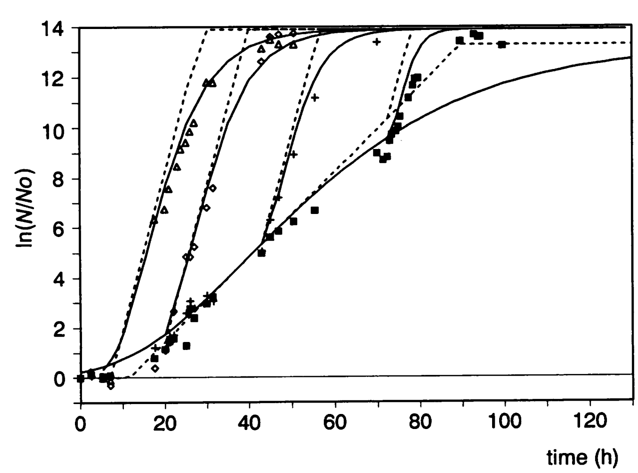

FIG. 8. Growth curves with shifts from 14 to 24°C compared with predictions with the modified Gompertz model with hypothesis 3 (——) and the linear model with hypothesis 2 (– – –). ■, E5; +, E7; ◇, E9; △, L67).

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APPENDIX

Prediction of bacterial growth at constant temperature. If the temperature remains constant, bacterial growth can be predicted by using the modified Gompertz equation (equation 2) and the growth parameters (A, μ, and λ) calculated from Table 1.

Equations for predicting microbial growth with temperature changes. (i) Use of hypothesis 3 (γ = 0.25). The lag time to be completed after a temperature shift within the lag phase ($t_s \leq \lambda_1$, $t_s^* \leq 1$) can be calculated with equation 6. With γ = 0.25, this results in:

$$\lambda_{\text{shift}} = (1 + \gamma - t_s^*) \cdot \lambda_2 = \left(1.25 - \frac{t_s}{\lambda_1}\right) \cdot \lambda_2 \qquad \text{(A-1)}$$

Subsequently, the modified Gompertz equation can be used to calculate the growth at times after the shift in temperature:

$$y = A_2 \exp\left\{- \exp\left[\frac{\mu_2 \cdot e}{A_2}(\lambda_{\text{shift}} - [t - t_s]) + 1\right]\right\} \qquad \text{(A-2)}$$

For a temperature shift within the exponential phase ($t_s > \lambda_1$, $t_s^* > 1$), before the time of shift, the modified Gompertz equation can be used to describe the bacterial number (y_1 is the logarithm of the relative number of organisms at the first temperature of incubation). The number of organisms at the time of shift [$y_1(t_s) = y_s$] can be predicted:

$$y_1(t) = A_1 \exp\left\{-\exp\left[\frac{\mu_1 \cdot e}{A_1}(\lambda_1 - t) + 1\right]\right\}$$
$$0 \le t \le t_s \tag{A-3}$$

After the shift in temperature, growth continues from y_s with an additional lag (λ_{shift}). y_2 is the logarithm of the relative number of organisms at the second temperature of incubation. This results in:

$$y_2(t) = y_s + (A_2 - y_s)$$
$$\exp\left\{-\exp\left[\frac{\mu_2 e}{A_2 - y_s}(\lambda_{shift} - [t - t_s]) + 1\right]\right\}$$
$$t > t_s \quad y_s = y_1(t_s) \quad \lambda_{shift} = y \cdot \lambda_2 = 0.25 \cdot \lambda_2 \tag{A-4}$$

(ii) Use of hypothesis 2 ($\gamma = 0$). The lag time to be completed after a temperature shift within the lag phase ($t_s \le \lambda_1$, $t_s^* \le 1$) can be calculated by assuming $\gamma = 0$ in equation 6:

$$\lambda_{shift} = (1 + \gamma - t_s^*) \cdot \lambda_2 = \left(1 - \frac{t_s}{\lambda_1}\right) \cdot \lambda_2 \tag{A-5}$$

Subsequently, the modified Gompertz equation (equation A-2) can be used to calculate the growth at times after the shift in temperature.

For a temperature shift within the exponential phase ($t_s > \lambda_1$, $t_s^* > 1$), the number of organisms before the time of shift can be predicted by equation A-3. The number of organisms after the time of shift can be predicted by:

$$y_2(t) = y_s + (A_2 - y_s)$$
$$\exp\left\{-\exp\left[\frac{\mu_2 e}{A_2 - y_s}(-[t - t_s]) + 1\right]\right\}$$
$$t > t_s \quad y_s = y_1(t_s) \tag{A-6}$$

(iii) Use of linear growth model and hypothesis 2 ($\gamma = 0$). The lag time to be completed after a temperature shift within the lag phase ($t_s \le \lambda_1$, $t_s^* \le 1$) can be calculated by equation A-5. After the lag time is completed, exponential growth will occur until the asymptote is reached:

$$y = 0 \quad t - t_s \le \lambda_{shift}$$
$$y = \mu_2 \cdot (t - t_s - \lambda_{shift})$$
$$\lambda_{shift} < t - t_s < A_2/\mu_2 + \lambda_{shift}$$
$$y = A_2 \; t - t_s \ge A_2/\mu_2 + \lambda_{shift} \tag{A-7}$$

For a temperature shift within the exponential phase ($t_s > \lambda_1$, $t_s^* > 1$), the number of organisms before the time of shift can be predicted by:

$$y = 0 \qquad t \le \lambda_1$$
$$y = \mu_1 \cdot (t - \lambda_1) \qquad \lambda_1 < t < t_s \tag{A-8}$$

and the growth after the time of shift (t_s) results in:

$$y = \mu_1 \cdot (t_s - \lambda_1) + \mu_2(t - t_s)$$
$$t_s \le t \le \frac{A_2 - \mu_1 \cdot (t_s - \lambda_1)}{\mu_2} + t_s \tag{A-9}$$
$$y = A2$$
$$t > \frac{A_2 - \mu_1 \cdot (t_s - \lambda_1)}{\mu_2} + t_s \tag{A-10}$$

This equation can be expanded very easily to more temperature shifts.

Various temperature shifts. If many temperature shifts occur or during dynamically changing temperature conditions, it will be better and easier to use hypothesis 2 ($\gamma = 0$) and the linear growth model. The modified Gompertz equation is far to complex to be used with many shifts.

Temperature shift within the lag phase. The lag time to be completed after many shifts within the lag phase can be calculated by assuming $\gamma = 0$ in equation 6 and adding all relative parts of the lag time that are completed until 1 is reached:

$$\Phi = \sum \frac{\Delta t_i}{\lambda_i} \text{ until } \Phi = 1 \tag{A-11}$$

where Φ is the sum of the relative parts of the lag time that are completed, Δt_i is the time that the organisms are at temperature i, and λ_i is the lag time at temperature i. For dynamically changing temperatures, this becomes:

$$\Phi = \int \frac{dt}{\lambda} \text{ until } \Phi = 1 \tag{A-12}$$

Temperature shift within the exponential phase. As soon as $\Phi = 1$, the exponential phase sets in. The time at which $\Phi = 1$ is defined as t_Φ:

$$y = 0 \qquad t \le t_\Phi$$
$$y = \mu_1 \cdot (t - t_\Phi) \qquad t_\Phi < t < t_{s1} \tag{A-13}$$

and the growth after the first time of shift (t_{s1}) in the exponential phase results in:

$$y = \mu_1(t_{s1} - t_\Phi) + \mu_2(t - t_{s1}) \qquad t_{s1} \le t \le t_{s2} \tag{A-14}$$

and the growth after the second time of shift (t_{s2}) in the exponential phase results in:

$$y = \mu_1(t_{s1} - t_\Phi) + \mu_2(t_{s2} - t_{s1}) + \mu_3(t - t_{s2})$$
$$t_{s2} < t < \frac{A - \mu_1(t_{s1} - t_\Phi) - \mu_2(t_{s2} - t_{s1})}{\mu_3} + t_{s2} \tag{A-15}$$

until the asymptote is reached:

$$\gamma = A \qquad t \ge \frac{A - \mu_1(t_{s1} - t_\Phi) - \mu_2(t_{s2} - t_{s1})}{\mu_3} + t_{s2} \tag{A-16}$$

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