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Revised model for aerobic growth of *Shigella flexneri* to extend the validity of predictions at temperatures between 10 and 19°C

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Abstract

Although *Shigella* is a major foodborne pathogen, its growth in foods has received little attention. Growth of *S. flexneri* 5348 inoculated into commercially available sterile foods (canned broths, meat, fish, UHT milk, baby foods) was studied at 10 to 37°C. *S. flexneri* was enumerated by surface-plating on Tryptic Soy Agar and growth curves were fitted by means of the Gompertz equation. Observed growth kinetics values and values calculated using a previously developed response surface model compared favorably for growth at 19 to 37°C, but not at < 19°C. To refine the model, additional data were collected for growth at 10 to 19°C. A total of 844 tests in BHI broth, representing 197 variable combinations of temperature (10–37°C), pH (5.0–7.5), NaCl (0.5–5.0%) and NaNO₂ (0–1000 ppm) was used for the revised model. The revised model, developed in BHI, gave significantly better agreement of calculated growth kinetics values with those observed in foods at 10 to 19°C. © 1998 Elsevier Science B.V.

Keywords: *Shigella flexneri*; Growth kinetics; Response surface models; Food; Temperature

1. Introduction

Shigella is a major cause of foodborne gastrointestinal illness (Smith, 1987). Its habitat is the intestinal tract of humans, as well as other primates. Epidemiological data most frequently implicate infected food handlers or fecally polluted water as contributing factors in foodborne shigellosis out-

breaks. Foods that are served raw, multiple ingredient foods (various salads) or cooked foods that are not reheated before serving are most often associated with shigellosis outbreaks. The potential for illness due to consumption of contaminated foods is relatively high, since the infective dose of *Shigella* is as low as 10–500 organisms (DuPont et al., 1989). Studies of *Shigella* inoculated into foods indicate that the organism is capable of surviving for extended periods under various conditions (Siegmond, 1960; Taylor and Nakamura, 1964). Recent reports

indicate that *Shigella* spp. grew readily when inoculated into foods: shredded cabbage (Satchell et al., 1990), sliced papaya and jicama (Fernandez Escartin et al., 1989), various sterile foods (Islam et al., 1993). However, little if any quantitative data on growth or survival of *Shigella* in food are available since many of the published reports provide few experimental details or are only descriptive in nature. A review of available information on growth and survival of *Shigella* spp. has been published recently (ICMSF, 1996).

Microbial growth models, based on growth kinetics data, are useful in providing an estimate of growth of a microorganism as a function of conditions relevant to the formulation, processing or storage of food. We have developed models which describe the effects and interactions of temperature, initial pH, sodium chloride and sodium nitrite on growth of *S. flexneri* in culture medium under aerobic (Zaika et al., 1992) and anaerobic conditions (Zaika et al., 1994). To validate these models we studied the growth characteristics of *S. flexneri* inoculated into various sterile foods and incubated at 10 to 37°C (Zaika and Scullen, 1996). The growth kinetics values observed in foods, expressed as T_{1000} (time, h, required for a 3 log increase in bacterial

population), agreed well with values calculated using the models for growth at 19 to 37°C, but not at < 19°C.

The objective of the present work was to obtain additional data for growth of *S. flexneri* at 10 to 19°C under aerobic conditions in brain–heart infusion (BHI) broth, to develop a new aerobic growth model, and to compare growth kinetics values observed in foods with those predicted by the models.

2. Materials and methods¹

2.1. Microorganism

Shigella flexneri 5348 (University of Texas Medical Branch, Galveston, TX, USA) was used throughout the study. To prepare the inoculum, the organism was cultured for 24 h in BHI broth (Difco Labs., Detroit, MI, USA) at 37°C, and the culture was diluted with sterile 0.1% peptone water.

2.2. Growth of *S. flexneri* in culture media

The procedure employed was described previously (Zaika et al., 1992). Briefly, sterile BHI media

Table 1
Gompertz equation and associated equations for growth kinetics values

	$L(t) = A + C \exp \{- \exp[-B(t - M)]\}$
$L(t)$	\log_{10} count of the number of bacteria at time t (h)
A	asymptotic log count as t decreases indefinitely (initial level of bacteria)
C	asymptotic amount of growth (log number) that occurs as t increases indefinitely (final log increase in bacterial numbers)
M	time (h) at which the culture achieves its maximum growth rate
B	relative growth rate at time M (h^{-1})
t	time (h)

Associated equations:

EGR	exponential growth rate $[(\log_{10} \text{ CFU/ml})/\text{h}] = BC/e$
LPD	lag phase duration (h) = $M - (1/B)$
GT	generation time (h) = $(\log_{10} 2)e/BC$
MPD	maximum population density ($\log_{10} \text{ CFU/ml}$) = $A + C$
T_{1000}	time (h) for population to increase from 1 to 1000 CFU/ml = $\{- \ln [- \ln (3/C)]\}/B + M$ = $\{1 - \ln [- \ln (3/C)]\}(GT)(C)/(\log_{10} 2)e + \text{LPD}$

Table 2

Gompertz equation parameters and derived growth kinetics values^a for *S. flexneri* cultured aerobically in BHI medium under various combinations of temperature, pH, sodium chloride and sodium nitrite concentrations

Temperature (°C)	pH	NaCl (%)	NaNO ₂ (ppm)	Rep	Gompertz parameters				EGR [(log ₁₀ CFU/ml)/h]	GT (h)	LPD (h)	MPD (log ₁₀ CFU/ml)
					A	C	B	M				
10	7.5	0.5	0	6 ^b	2.99	NG ^c	0.0000	0.000				
10	7.0	0.5	0	3	3.07	NG	0.0000	0.000				
10	7.0	2.5	0	3	3.21	NG	0.0000	0.000				
10	6.5	0.5	0	5 ^b	3.15	5.43	0.0101	452.10	0.020	17.4	338.7	8.6
10	6.5	2.5	0	3	3.17	NG	0.0000	0.000				
10	6.0	0.5	0	3	3.04	5.91	0.0075	228.18	0.016	18.5	94.4	9.0
10	6.0	2.5	0	3	3.09	NG	0.0000	0.000				
10	5.5	0.5	0	3 ^b	3.09	NG	0.0000	0.000				
10	5.5	2.5	0	3	3.05	NG	0.0000	0.000				
12	7.5	0.5	0	3 ^b	3.76	6.13	0.0105	406.96	0.024	12.9	310.2	9.9
12	7.0	0.5	0	1	4.00	5.87	0.0143	379.56	0.031	9.8	309.6	9.9
12	6.5	0.5	0	6 ^b	3.60	5.80	0.0116	289.35	0.024	13.0	196.1	9.4
12	6.5	2.5	0	3	3.18	NG	0.0000	0.000				
12	6.0	0.5	0	3 ^b	3.51	5.81	0.0132	168.60	0.028	10.8	92.1	9.3
12	6.0	2.5	0	3	3.22	NG	0.0000	0.000				
12	5.5	0.5	0	3 ^b	3.47	5.45	0.0071	295.59	0.014	21.2	154.6	8.9
12	5.5	2.5	0	3	3.25	NG	0.0000	0.000				
12	5.0	0.5	0	3	3.08	NG	0.0000	0.000				
12	5.0	2.5	0	3	3.24	NG	0.0000	0.000				
15	7.5	0.5	0	3 ^b	2.82	7.35	0.0372	42.04	0.101	3.0	15.1	10.2
15	7.5	2.5	0	3	3.30	6.46	0.0204	152.90	0.049	6.4	102.4	9.8
15	7.5	4.0	0	3	2.92	NG	0.0000	0.000				
15	7.0	0.5	0	3	2.85	7.43	0.0384	51.14	0.105	2.9	24.8	10.3
15	7.0	2.5	0	3	3.33	6.44	0.0253	123.34	0.059	5.2	82.4	9.8
15	7.0	4.0	0	3	2.91	NG	0.0000	0.000				
15	6.5	0.5	0	3 ^b	2.82	7.28	0.0387	38.43	0.104	2.9	12.6	10.1
15	6.5	2.5	0	3	3.47	6.11	0.0223	113.90	0.050	6.1	68.6	9.6
15	6.5	4.0	0	3	2.65	5.68	0.0065	581.14	0.014	22.8	423.0	8.3
15	6.0	0.5	0	6 ^b	3.05	7.04	0.0368	40.07	0.096	3.2	12.8	10.1
15	6.0	0.5	50	3	3.12	6.39	0.0299	54.14	0.070	4.3	18.6	9.5
15	6.0	0.5	100	3	3.42	5.98	0.0263	81.61	0.058	5.2	43.6	9.4
15	6.0	0.5	200	3	3.28	5.09	0.0102	210.77	0.019	16.0	111.2	8.4
15	6.0	2.5	0	6	3.72	5.68	0.0245	160.68	0.051	6.1	118.5	9.4
15	6.0	2.5	50	1 ^d	2.93	3.99	0.0104	432.02	0.015	19.7	335.9	6.9
15	6.0	2.5	100	3	3.05	NG	0.0000	0.000				
15	6.0	2.5	200	3	3.04	NG	0.0000	0.000				
15	6.0	4.0	0	3	2.83	NG	0.0000	0.000				
15	5.5	0.5	0	3 ^b	2.91	6.76	0.0256	58.10	0.064	4.7	19.0	9.7
15	5.5	2.5	0	3	3.08	5.03	0.0112	467.60	0.020	15.0	375.0	8.1
15	5.5	4.0	0	3	2.82	NG	0.0000	0.000				
15	5.0	0.5	0	3	3.01	5.97	0.0126	108.98	0.028	10.9	29.6	9.0
15	5.0	2.5	0	3	3.18	NG	0.0000	0.000				
15	5.0	4.0	0	3	2.80	NG	0.0000	0.000				
19	7.5	4.0	0	3 ^b	2.95	NG	0.0000	0.000				
19	7.0	4.0	0	3	3.11	NG	0.0000	0.000				
19	6.5	4.0	0	3 ^b	3.17	NG	0.0000	0.000				
19	6.0	0.5	0	6	3.14	7.03	0.0495	31.70	0.128	2.4	11.4	10.2
19	6.0	0.5	50	3	3.21	6.61	0.0444	38.89	0.108	2.8	16.4	9.8
19	6.0	0.5	100	3	3.23	6.28	0.0265	52.12	0.061	4.9	14.4	9.5
19	6.0	0.5	200	3	3.23	NG	0.0000	0.000				

Table 2 (continued)

Gompertz equation parameters and derived growth kinetics values^a for *S. flexneri* cultured aerobically in BHI medium under various combinations of temperature, pH, sodium chloride and sodium nitrite concentrations

19	6.0	2.5	0	21	3.44	6.13	0.0258	97.35	0.058	5.3	57.2	9.6
19	6.0	2.5	50	3	3.61	5.27	0.0240	229.78	0.047	7.6	181.3	8.9
19	6.0	2.5	100	3 ^e	3.52	5.47	0.0219	222.54	0.044	6.9	176.7	9.0
19	6.0	2.5	200	3	3.15	NG	0.0000		0.000			
19	6.0	4.0	0	1 ^d	2.54	6.66	0.0066	604.92	0.016	18.8	453.4	9.1
19	5.5	0.5	0	3 ^b	3.09	6.77	0.0361	37.80	0.090	3.4	10.0	9.9
19	5.5	2.5	0	3	2.78	5.69	0.0060	533.64	0.013	24.8	363.6	8.5
19	5.5	4.0	0	3	3.04	NG	0.0000		0.000			
19	5.0	0.5	0	6	3.20	6.01	0.0168	90.96	0.037	8.2	30.8	9.2
19	5.0	0.5	50	3	3.44	NG	0.0000		0.000			
19	5.0	0.5	100	3	3.42	NG	0.0000		0.000			
19	5.0	0.5	200	3	3.59	NG	0.0000		0.000			
19	5.0	2.5	0	3	3.02	NG	0.0000		0.000			
19	5.0	4.0	0	3	3.05	NG	0.0000		0.000			
28	5.0	0.5	0	7	3.27	7.23	0.1229	20.24	0.327	0.9	11.9	10.5
28	5.0	0.5	50	6	3.29	6.92	0.0801	88.31	0.207	1.7	74.6	10.2
28	5.0	0.5	100	5	3.04	6.98	0.0590	201.68	0.151	2.0	184.4	10.0
28	5.0	0.5	200	3	3.21	NG	0.0000		0.000			

^a See Table 1 for definition of terms.

^b Additional data reported previously.

^c NG = No growth.

^d Growth was obtained in one of three replicates.

^e Growth was obtained in three of six replicates.

having the desired pH, NaCl and NaNO₂ content were inoculated with *S. flexneri* to an initial level of 10³ CFU/ml and incubated on a rotary shaker (150 rpm) at the desired temperature. Bacterial populations were determined at appropriate time intervals by surface-plating the cultures or their dilutions on Tryptic Soy Agar (Difco).

2.3. Growth of *S. flexneri* in foods

Growth studies using sterile commercially available foods were conducted as described previously (Zaika and Scullen, 1996). The sodium chloride concentrations were calculated from the levels of sodium listed on the manufacturers' labels. Determination of the pH values of the liquid foods was carried out by direct measurement. Determination of the pH values of baby foods and canned foods were done using slurries prepared with distilled water to 1 to 5 and 1 to 10 dilutions, respectively. Liquid foods, 100 ml, were inoculated with *S. flexneri* to an initial level of 10³ CFU/ml; baby foods and canned foods, 100 g, were inoculated to an initial level of 10⁴ CFU/g. The inoculated foods were incubated at the

desired temperature and bacterial populations determined as described above.

2.4. Curve fitting

Growth curves were generated from bacterial population data using the Gompertz equation (Gibson et al., 1988) (Table 1) in conjunction with ABACUS, a nonlinear regression program that employs a Gauss-Newton iteration procedure (Damert, 1994). The Gompertz parameter values (*A*, *B*, *C*, *M*) were used to calculate exponential growth rates (EGRs) [(log₁₀ CFU/ml)/h], generation times (GTs) (h), lag phase durations (LPDs) (h), maximum population densities (MPDs) (log₁₀ CFU/ml) and times for populations to increase from 1 to 1000 CFU/ml (*T*₁₀₀₀) (h) as previously described by Gibson et al. (1988) and Buchanan and Klawitter (1992).

2.5. Statistical analysis

The new growth data were combined with data used to develop the original growth model (Zaika et

al., 1992). Second- and third-order response surface models in terms of temperature, pH, sodium chloride concentration and sodium nitrite concentration were calculated for the *S. flexneri* growth data using least-squares analysis of PROC GLM of the SAS system (SAS Institute, 1989, pp. 871–996). The regression analysis was performed on the Ln transformations (Gibson et al., 1988) of the Gompertz parameters *B* and *M* and of the kinetics values GT and LPD. Second- and third-order models for the Ln transformations were also subjected to stepwise regression using backward elimination model selection technique (Draper and Smith, 1981, pp. 305–307) with $\alpha = 0.1$ as significance level for retention of terms.

3. Results

To extend the validity of the aerobic growth model (Zaika et al., 1992) to low temperatures, additional growth data were obtained for *S. flexneri* cultured in BHI media (Table 2). Sodium chloride exerted a significant inhibitory effect on the bacterium at temperatures $< 19^{\circ}\text{C}$, while optimum growth occurred at pH 6.0 and 6.5. In the presence of 0.5% NaCl the bacterium grew at 12°C in media of pH 7.5 to 5.5 and at 10°C at pH 6.5 and 6.0; however, growth did not occur in the presence of 2.5% NaCl. Growth occurred at pH 5.0 at 15 and 19°C , but only at the lowest NaCl concentration tested. At 19°C , sodium nitrite prevented growth of the organism at pH 5.0 but not at pH 6.0. Additional observations on growth of *S. flexneri* at 28°C were also included in the data base (Table 2).

A total of 844 tests, representing 197 variable combinations were available for model development. Growth was observed in 519 tests, representing 125 variable combinations. These growth data were used to derive the revised models for aerobic growth of *S. flexneri*. A total of four second-order and four third-order Ln transformation-based models were generated (Table 3). The Ln transformation is useful in stabilizing the variances of the experimental values (Gibson et al., 1988; Alber and Schaffner, 1992). Similar models were obtained previously (Zaika et al., 1994) for anaerobic growth of *S. flexneri*. Model 1 is analogous to the original aerobic growth model (Zaika et al., 1992). Previous attempts to incorporate

Table 3
Response surface models^a for aerobic growth of *S. flexneri* in BHI broth

	R^2	No. of terms
<i>Second-order models</i>		
Model 1		
Ln (<i>B</i>)	0.864	15
Ln (<i>M</i>)	0.847	15
Model 2		
Ln (<i>B</i>) after SRBE ^b	0.863	12
Ln (<i>M</i>) after SRBE	0.847	13
Model 3		
Ln (GT)	0.862	15
Ln (LPD)	0.755	15
Model 4		
Ln (GT) after SRBE	0.861	13
Ln (LPD) after SRBE	0.754	12
<i>Third-order models</i>		
Model 5		
Ln (<i>B</i>)	0.890	35
Ln (<i>M</i>)	0.893	35
Model 6		
Ln (<i>B</i>) after SRBE	0.887	23
Ln (<i>M</i>) after SRBE	0.892	25
Model 7		
Ln (GT)	0.893	35
Ln (LPD)	0.837	35
Model 8		
Ln (GT) after SRBE	0.892	23
Ln (LPD) after SRBE	0.834	25

^a 519 observations were used for each model; no growth responses were treated as missing values.

^b Stepwise regression–backward elimination method (with $\alpha = 0.10$).

no-growth data into model development did not improve the fit (Zaika et al., 1992, 1994). The R^2 values for all eight models were satisfactory, and the stepwise regression–backward elimination procedure did not affect them significantly while eliminating a large number of non-significant terms from the third-order models (Table 3).

Additional experiments were carried out to determine the growth characteristics of *S. flexneri* inoculated into various sterile foods and incubated at 10 to 15°C (Table 4). Several brands of canned beef broths and chicken broths used previously (Zaika and Scullen, 1996) were included in the present study. The symbols A_1 , A_2 and B_1 , B_2 represent changes in formulation as listed on the product label.

Table 4
Gompertz equation parameters and derived growth kinetics values^a for *S. flexneri* cultured in foods

	Temperature (°C)	pH	NaCl (%)	Rep	Gompertz parameters			EGR [(log ₁₀ CFU/ml)/h]	GT (h)	LPD (h)	MPD (log ₁₀ CFU/ml)	
					A	B	M					
<i>Canned broths</i>												
Beef broth A	15	5.90	0.87	3	2.96	5.24	0.0720	29.12	0.138	2.3	14.6	8.2
Beef broth A	10	5.90	0.87	6	3.70	5.38	0.0189	102.10	0.037	8.2	48.9	9.1
Beef broth B ₁	15	6.05	0.69	3	2.97	NG ^b	0.0000		0.000			
Beef broth B ₂	15	7.13	0.66	6	3.62	5.36	0.0368	46.42	0.072	4.2	18.6	9.0
Beef broth C	15	6.10	1.38	3	3.18	NG	0.0000		0.000			
Chicken broth A ₁	15	6.70	0.68	3	3.15	6.31	0.0496	36.78	0.115	2.6	16.6	9.5
Chicken broth A ₁	10	6.70	0.68	3	3.92	NG	0.0000		0.000			
Chicken broth A ₂	15	6.47	0.68	3	2.98	5.46	0.0552	28.98	0.111	2.7	10.8	8.4
Chicken broth B	10	6.43	1.11	3	3.97	NG	0.0000		0.000			
Chicken broth C	15	6.37	0.12	3	3.18	5.21	0.0502	34.15	0.096	3.1	15.2	8.4
Chicken broth C	10	6.37	0.12	6	3.33	4.82	0.0239	211.59	0.041	10.6	150.0	8.2
Vegetable broth	15	5.66	1.06	3	3.04	4.74	0.0190	181.43	0.033	9.2	127.8	7.8
<i>Baby foods</i>												
Squash	12	5.37	0.02	3	3.70	NG	0.0000		0.000			
Veal and gravy	15	5.98	0.12	3	4.03	4.79	0.0582	43.23	0.102	2.9	26.0	8.8
<i>Other foods</i>												
Canned salmon A	15	6.35	1.09	3	3.35	6.05	0.0190	66.88	0.042	7.1	14.1	9.1
Milk, UHT	10	6.50	0.14	3	3.57	NG	0.0000		0.000			

^a See Table 1 for definition of terms.

^b NG = No growth.

The organism is capable of growth at 10°C. It grew well in beef broth A and chicken broth C but not in UHT milk or chicken broths A₁ or B. Good growth was obtained in chicken broths, beef broths A and B₂, canned pink salmon and baby food veal at 15°C. Growth was slow in vegetable broth at 15°C and did not occur in baby food squash at 12°C. At least to some extent, differences in growth characteristics appeared to be brand-dependent, which may be due to differences in product formulations. For example, while beef broths A and B₁ do not differ significantly in pH and sodium chloride content, they differ in the type and/or quantity of food additives. *S. flexneri* grew well in beef broth A at 15°C and even at 10°C; however, it failed to grow in beef broth B₁. Little, if any information is available on the influence on microbial growth of various ingredients, such as protein hydrolysates, that are commonly added to foods.

Three of the models for aerobic growth of *S. flexneri* obtained were selected for evaluation (Table 5). The Gompertz parameters of $A = 2.97$ and $C = 6.21$, the grand means of the experimental data for BHI media, were used in conjunction with B and M terms predicted by the models to calculate predicted GT, LPD and T_{1000} values (Table 1). Growth kinetics values, expressed as T_{1000} (time required for lag plus a 3 log increase in bacterial population), observed in foods were compared with those pre-

dicted by the models (Table 6). Data for foods obtained previously (Zaika and Scullen, 1996) are included in the evaluation. M1, M3 and M6 are the new models 1, 3 and 6, respectively, obtained using the expanded data set. M is the previously described model (Zaika et al., 1992) and is analogous to M1.

All four models gave a reasonable estimate of *S. flexneri* growth in foods at 19 to 37°C. Although agreement between observed and predicted growth kinetics values was not as good for temperatures < 19°C, considerably better agreement was obtained for values calculated using models 1, 3 or 6 compared to the original model (M). Most often the closest agreement of the observed T_{1000} values was obtained with calculated values using model 3. In a number of instances *S. flexneri* failed to grow during a minimum of 400 h of incubation, or died off to undetectable levels, although growth was predicted by models 1, 3 or 6. This usually occurred when the inoculated food was incubated at low temperatures (15°C or below). However, the bacteria inoculated into carrots failed to grow and died off rapidly even at 19 and 28°C. Antimicrobial compounds, either constitutive or induced, are known to occur in plants. For example, Kurosaki and Nishi (1983) showed that 6-methoxymellein, the stress-induced metabolite from carrot roots, effectively inhibited the growth of fungi and gram-positive bacteria. However, gram-negative bacteria, including *S. sonnei*, were less

Table 5

Three response surface models found to be effective for describing the effects and interactions of temperature (T , °C), initial pH (P), sodium chloride concentration (S , %) and sodium nitrite concentration (N , ppm) on the aerobic growth of *S. flexneri*

Model 1.	Full regression second-order models of Gompertz B and M values
$\text{Ln}(B) =$	$-15.3753 + 0.29408T + 2.2611P - 0.115S - 0.009789N + 0.00146TP + 0.001919TS + 0.00005048TN - 0.00107PS + 0.0009225PN + 0.0002196SN - 0.003403T^2 - 0.16224P^2 - 0.039505S^2 + 0.00000094N^2$
$\text{Ln}(M) =$	$+28.7462 - 0.3395T - 6.0634P + 0.05237S + 0.02366N - 0.007044TP - 0.01342TS - 0.00010126TN + 0.05153PS - 0.002604PN + 0.00004229SN + 0.004894T^2 + 0.46462P^2 + 0.072619S^2 - 0.00000138N^2$
Model 3.	Full regression second-order models of growth kinetics values for GT and LPD
$\text{Ln}(GT) =$	$15.6043 - 0.3833T - 2.7043P + 0.4172S + 0.01155N + 0.00533TP - 0.00567TS - 0.0000789TN - 0.0376PS - 0.001087PN - 0.000153SN + 0.004387T^2 + 0.18615P^2 + 0.06413S^2 - 0.0000007N^2$
$\text{Ln}(LPD) =$	$38.6743 - 0.4091T - 9.227P + 0.0948S + 0.033N - 0.004979TP - 0.02099TS - 0.0001319TN + 0.07073PS - 0.003767PN + 0.000152SN + 0.005737T^2 + 0.7092P^2 + 0.1046S^2 - 0.0000014N^2$
Model 6.	Stepwise regression-backward elimination third-order models of Gompertz B and M values
$\text{Ln}(B) =$	$-46.7179 - 0.7058T + 21.2137P - 0.6757S + 0.0699N + 0.1677TP - 0.00069TN - 0.02225PN + 0.005668SN + 0.01643T^2 - 3.4915P^2 + 0.2396S^2 + 0.0000184N^2 - 0.000259T^3 + 0.1905P^3 - 0.01291P^2T + 0.00159P^2N - 0.0345S^3 - 0.00000011N^2T - 0.00000203N^2P + 0.0001278TPN - 0.0000388TSN - 0.000603PSN$
$\text{Ln}(M) =$	$+133.9337 - 61.3563P + 10.3365S + 0.1742N - 0.06422TP - 0.1063TS + 0.00099TN - 2.5904PS - 0.0501PN - 0.00101SN + 9.8045P^2 - 0.0000118N^2 + 0.0000501T^3 + 0.000808T^2S - 0.5147P^3 + 0.004248P^2T + 0.1666P^2S + 0.003878P^2N - 0.02415S^3 + 0.0391S^2P + 0.00000035N^2T + 0.00000053N^2S + 0.00743TPS - 0.0002026TPN + 0.0000226TSN$

Table 6
Comparison of growth of *S. flexneri* observed in foods with growth predicted by models

	pH	NaCl (%)	Temperature (°C)	T_{1000} (h)					
				Observed	Predicted ^a				
					M	M1	M3	M6	
<i>Canned broths</i>									
Beef broth A	5.90	0.87	37	5.4	6.3	6.4	5.8	4.9	
			19	29.5	105.9	53.7	48.7	45.8	
			15	37.2	416.2	129.9	121.4	105.4	
			12	92.4	> 1000	278.8	269.0	205.8	
Beef broth B ₁	6.05	0.69	10	130.6	> 1000	486.8	483.4	328.5	
			37	8.0	5.7	5.8	5.3	4.8	
			19	62.4	92.1	47.5	43.3	44.0	
			15	NG	359.4	114.3	106.3	99.7	
Beef broth B ₂	7.13	0.66	12	NG	> 1000	244.2	233.1	192.0	
			15	61.2	228.9	110.0	97.3	137.9	
Beef broth C	6.10	1.38	37	7.8	5.6	5.8	5.6	5.0	
			19	80.7	93.8	55.0	50.4	54.0	
			15	NG	370.2	137.1	128.6	132.6	
Chicken broth A ₁	6.70	0.68	19	38.8	65.1	40.7	36.8	55.8	
			15	42.8	244.6	99.5	90.0	128.8	
			10	NG	> 1000	377.2	352.4	404.0	
Chicken broth A ₂	6.47	0.68	15	38.7	269.5	100.2	91.8	116.6	
Chicken broth B	6.43	1.11	10	NG	> 1000	441.4	423.2	429.1	
Chicken broth C	6.37	0.12	15	47.0	280.9	90.2	83.7	103.1	
			10	242.8	> 1000	326.6	312.3	291.9	
Vegetable broth	5.66	1.06	37	12.7	7.5	7.9	6.7	5.7	
			19	231.9	136.0	66.7	60.5	53.5	
			15	222.6	544.1	162.0	153.2	124.9	
			12	NG	> 1000	348.8	344.6	247.7	
<i>Baby foods</i>									
Carrots	5.05	0.22	28	NG	38.0	30.4	23.8	22.2	
			19	NG	334.9	114.5	101.2	78.5	
Chicken and broth	6.43	0.20	37	6.2	5.0	5.0	4.9	5.7	
			15	43.5	269.1	90.4	83.6	107.2	
Peas	6.51	0.00	15	54.4	258.0	86.0	79.9	113.0	
Squash	5.37	0.02	28	14.8	24.8	20.0	16.1	12.4	
			15	231.1	829.8	172.7	159.5	90.0	
			12	NG	> 1000	356.5	346.4	156.8	
Veal and gravy	5.98	0.12	15	56.3	384.8	105.5	97.9	84.2	
<i>Canned meat/fish</i>									
Dog food, beef	6.85	1.50	28	9.8	9.5	11.0	10.2	13.1	
			19	32.3	71.3	52.3	46.2	67.0	
			12	159.5	881.9	299.9	275.8	353.8	
Salmon A ₁	6.44	1.22	28	12.8	9.9	10.6	9.9	11.1	
Salmon A ₂	6.35	1.09	15	85.5	300.2	115.2	106.1	124.3	
Salmon B	6.80	1.10	12	373.8	813.6	252.0	230.1	296.2	
Lunchmeat A	6.50	2.68	28	34.1	13.3	15.8	15.0	16.0	
Lunchmeat B	6.69	2.51	19	NG	98.0	77.3	70.5	95.2	
<i>Dairy products</i>									
Milk, UHT	6.50	0.14	28	10.4	9.4	9.5	8.7	12.2	
			19	53.4	69.2	39.7	34.3	51.2	
			15	76.0	259.9	95.5	81.7	111.7	
			12	257.0	839.8	204.1	174.5	208.3	
			10	NG	> 1000	355.2	305.4	320.2	

sensitive to the compound. Beuchat and Brackett (1990) observed that populations of viable *Listeria monocytogenes* decreased on contact with raw carrots but not cooked carrots. Thus, the presence and stability of antimicrobial constituents in foods must be considered when using predictive models. No systematic bias was noted between the observed growth kinetics values and those predicted by models 1, 3 or 6. In some foods the potential for growth was underestimated, while for others it was overestimated (Table 6). For example, the bacterium grew consistently faster in beef broth A and consistently slower in beef broths B₁ and C than predicted by the models for all the temperatures.

4. Discussion

We developed several response surface models for aerobic growth of *S. flexneri* using an expanded data base. All three models chosen for evaluation gave better prediction of bacterial growth compared to the previously obtained model. Model 3 was selected for inclusion into the USDA Pathogen Modeling Program Version 5.0, since it permits calculation of an error matrix and determination of 95% confidence limits. Use of the modeling program to predict growth of *S. flexneri* is shown in Fig. 1. Under favorable conditions, 37°C, for beef broth A the observed T_{1000} (5.4 h) is well within the confidence

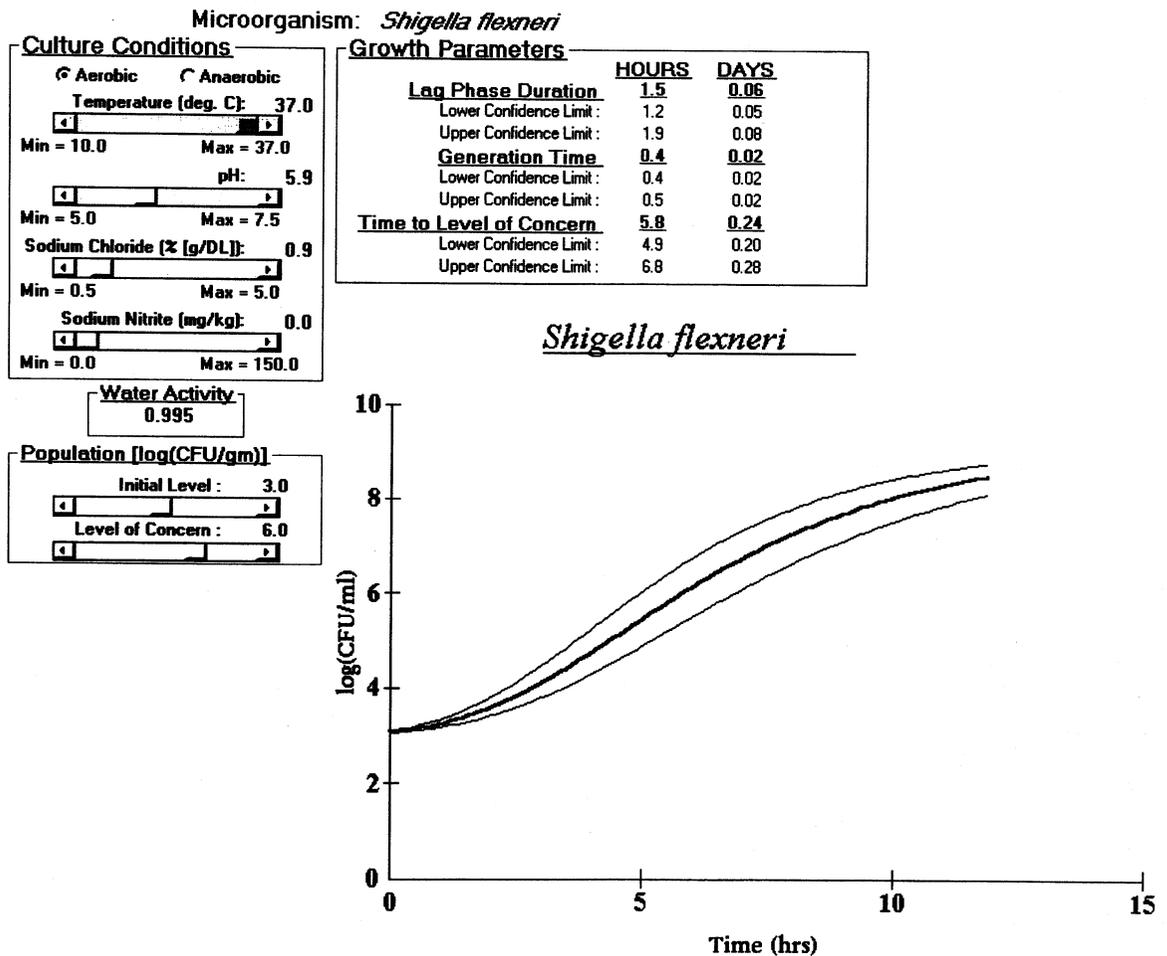
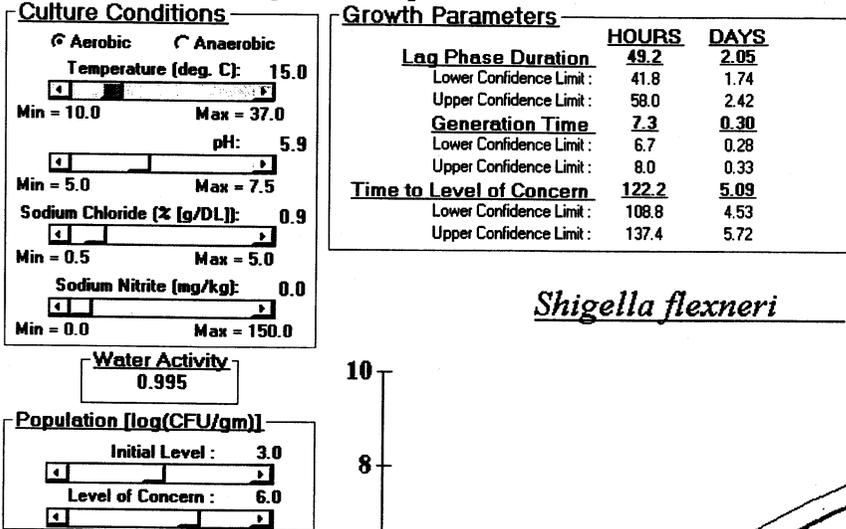


Fig. 1. Use of the USDA Pathogen Modeling Program Version 5.0 to predict growth of *S. flexneri* in beef broth A at 37°C (a) and at 15°C (b). Figures illustrate the software format with environmental and parameter entry on the left, predicted growth parameters on the right and predicted growth curve below.

Microorganism: *Shigella flexneri*



Shigella flexneri

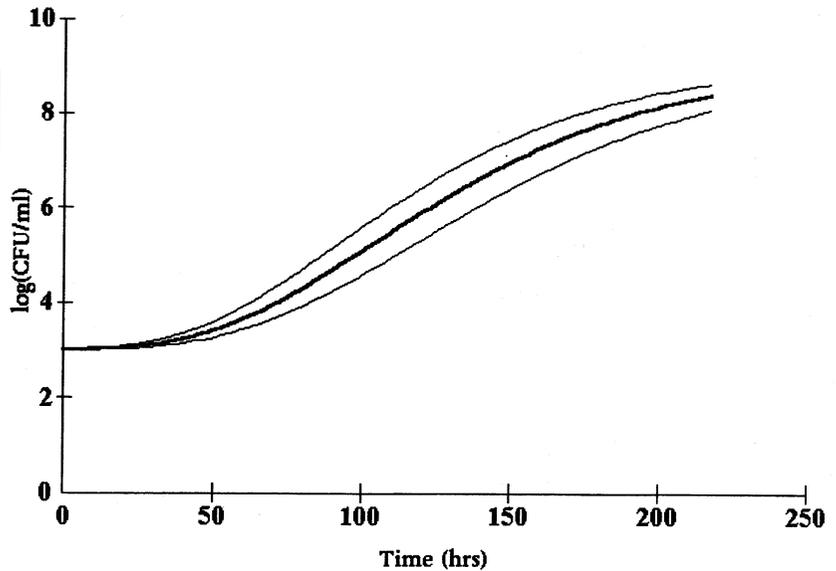


Fig. 1. (continued)

limits given by the model (4.9–6.8) (Fig. 1a). However, under unfavorable conditions, 15°C, the observed T_{1000} value (37.2 h) is considerably shorter than the predicted value (108.8–137.4) (Fig. 1b).

The model is intended to be used as a means of estimating growth of the organism in foods under conditions within the boundaries of the range of the variables studied. The model is based on only four variables: temperature, pH, sodium chloride and sodium nitrite concentrations. Our results suggest that, although these parameters, particularly temperature, are of major importance for bacterial growth, other factors may play a significant role. The influence of components characteristic of a given food or commonly used food additives should be taken into account in model development to obtain better

agreement of observed and predicted growth kinetics values. Also, as conditions become more adverse, bacterial growth becomes more variable. This is reflected in an increase in the confidence limit ranges for growth under inhibitory conditions compared to growth under optimum conditions.

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