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Model for the combined effects of temperature, initial pH, sodium chloride and sodium nitrite concentrations on anaerobic growth of *Shigella flexneri*

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Abstract

A fractional factorial design was used to measure the effects and interactions of temperature (12–37°C), initial pH (5.5–7.5), NaCl (0.5–4.0%) and NaNO₂ (0–1000 ppm) on the anaerobic growth kinetics of *Shigella flexneri* in Brain-Heart Infusion broth. Anaerobic conditions were established by flushing the culture flasks with N₂. A total of 375 cultures representing 124 variable combinations were analyzed, with growth curves being generated using the Gompertz equation. Growth rates decreased with decreasing temperature, decreasing pH and increasing NaCl level. NaNO₂ in combination with low temperature, low pH and high NaCl content effectively inhibited *S. flexneri*. Response surface analysis was used to obtain models for estimating the growth of *S. flexneri* in terms of temperature, initial pH, and NaCl and NaNO₂ concentrations. A third-order equation using the natural logarithm transformations for the Gompertz *B* and *M* terms gave reasonable estimates of bacterial growth in response to any combination of the variables studied within the specified ranges.

Keywords: *Shigella flexneri*; Anaerobic growth kinetics; Modelling; Response surface model; Temperature

1. Introduction

Shigella is an important causative agent of gastrointestinal illness (Smith, 1987). In many parts of the world shigellosis is endemic and is complicated by a high degree of multiple drug resistance. Epidemiological data most frequently implicate poor personal hygiene as a contributing factor in foodborne shigellosis outbreaks (Bean and Griffin, 1990). The infectious dose appears to be very low, 10–500 organisms, based on human volunteer studies (DuPont et al., 1989). Bean and Griffin (1990) reported that in the United States *Shigella* spp. were identified in 104 outbreaks (14 399 cases) out of 1869 outbreaks (108 906 cases) of known etiology involving bacteria. Estimates of 163 000 shigellosis cases per year (Todd, 1989) and as high as 440 000 cases per year (Archer and Kvenberg, 1985) have been suggested. Some recent shigellosis outbreaks were associated with commercially shredded lettuce (Davis et al., 1988), German potato salad (Lew et al., 1991) and uncooked tofu salad (Lee et al., 1991).

Although many studies have been reported on the taxonomy, epidemiology and virulence of *Shigella* spp., much less attention has been devoted to the growth characteristics of the organism (Fehlhaber, 1981). Reports of *Shigella* in food are limited, but it appears that the organism is capable of surviving for extended periods in different types of foods under various conditions (Satchell et al., 1990; Siegmund, 1960; Taylor and Nakamura, 1964). It is necessary to understand the effect of conditions in food on the growth of *Shigella* to estimate its potential for growth in food and to identify factors that play a major role in controlling its proliferation.

In previous studies we have developed a model describing the combined effects of temperature, initial pH, sodium chloride and sodium nitrite on growth of *S. flexneri* in a culture medium under aerobic conditions (Zaika et al., 1989, 1991, 1992). The objective of the present work was to develop a model for the combined effects of these factors on growth under conditions of limited availability of oxygen.

2. Materials and methods

Microorganism. *Shigella flexneri* 5348 (obtained from Dr. David W. Niesel, University of Texas Medical Branch, Galveston, TX) was used throughout the study. To prepare the inoculum, the organism was cultured for 24 h in Brain-Heart Infusion (BHI, Difco Laboratories, Detroit, MI) at 37°C, and the culture was diluted with sterile 0.1% peptone water.

Experimental design. An incomplete factorial design was used to assess the effects of temperature (12, 15, 19, 28, 37°C), initial pH (5.5, 6.0, 6.5, 7.0, 7.5), sodium chloride (0.5, 2.5, 4.0%) and sodium nitrite (0, 50, 100, 200, 1000 ppm). The number of replicate cultures tested for each variable combination is given in Table 2.

Culture techniques. BHI, prepared to 90% of final volume, was supplemented with 0, 20 or 35 g sodium chloride/900 ml. pH was adjusted to 5.5, 6.0, 6.5, 7.0 or 7.5 using 1 N NaOH or 1 N HCl. The media were then dispensed in 45-ml portions into 250-ml trypsinizing flasks with the sidearm port fitted with a rubber septum and sterilized by autoclaving for 15 min at 121°C. Filter-sterilized sodium nitrite solutions (0, 500, 1000, 2000 or 10000 ppm) were added in 5-ml volumes to the sterile media. The final media contained 0.5, 2.5 or 4.0% (w/v) sodium chloride and 0, 50, 100, 200 or 1000 ppm (micrograms/ml) sodium nitrite.

All media were inoculated with 0.5 ml of a diluted 24-h culture of *S. flexneri* to an initial level of approximately 1×10^3 cfu/ml. The flasks were then flushed with sterile nitrogen for 15 min, sealed with a sterile rubber stopper and incubated on a rotary shaker (150 rpm) at the desired temperature.

At appropriate time intervals samples were withdrawn from each flask by means of a syringe and the microbial population determined by surface plating of appropriate dilutions on Tryptic Soy Agar (Difco) using a Spiral Plater (Spiral System Instruments, Inc., Bethesda, MD). The plates (in duplicate) were incubated for 24 h at 37°C and counted.

Curve fitting. Growth curves were generated from the experimental data using the Gompertz equation (Table 1) in conjunction with ABACUS, a nonlinear regression program that employs a Gauss-Newton iteration procedure. This FORTRAN-based program was developed by W.C. Damert (Eastern Regional Research Center, U.S. Department of Agriculture, Philadelphia, PA), and copies are available upon request. The Gompertz parameter values (A , B , C , M) were subsequently used to calculate lag times (h), exponential growth rates [$(\log_{10}$ cfu/ml)/h], generation times (h) and maximum population densities (\log_{10} cfu/ml) as described by Gibson et al. (1987) (Table 1).

Table 1
Gompertz equation and associated equations for growth kinetics values

Gompertz equation

$$L(t) = A + C \exp(-\exp(-B(t - M)))$$

$L(t)$ \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 = BC/e [\log_{10} (cfu/ml)/h]

GT generation time = $(\log_{10} 2)e/(BC)$ (h)

LPD lag phase duration = $M - (1/B)$ (h)

MPD maximum population density = $A + C$ [\log_{10} (cfu/ml)]

Table 2

Gompertz equation parameters and derived growth kinetics values for *Shigella flexneri* cultured anaerobically in BHI medium under various combinations of temperature, pH, sodium chloride and sodium nitrite concentrations

Temperature	pH	NaCl (%)	NaNO ₂ (ppm)	REP	Gompertz parameters				EGR (Log/h)	GT (h)	LPD (h)	MPD (Log)
					A	C	B	M				
12	7.5	0.5	0	3	2.85	NG	0.0000		0.000			
12	6.5	0.5	0	3	2.82	NG	0.0000		0.000			
12	5.5	0.5	0	3	2.91	NG	0.0000		0.000			
15	7.5	0.5	0	3	3.53	5.06	0.0131	217.85	0.022	14.10	127.61	8.59
15	7.5	0.5	50	1	2.20	7.05	0.0149	243.86	0.039	7.79	176.75	9.25
15	7.5	2.5	0	2	2.90	NG	0.0000		0.000			
15	7.5	2.5	200	2	2.89	NG	0.0000		0.000			
15	7.0	0.5	0	3	3.57	5.06	0.0076	253.10	0.014	21.56	120.63	8.62
15	7.0	0.5	50	2	2.26	6.01	0.0057	439.14	0.013	24.30	261.51	8.27
15	7.0	2.5	0	2	3.11	5.62	0.0147	343.81	0.030	10.04	274.68	8.73
15	7.0	2.5	200	2	2.81	NG	0.0000		0.000			
15	6.5	0.5	0	3	3.19	5.47	0.0161	189.38	0.032	9.54	125.63	8.66
15	6.5	0.5	50	2	2.65	NG	0.0000		0.000			
15	6.0	0.5	0	3	2.93	6.18	0.0154	207.44	0.035	8.64	142.37	9.11
15	5.5	0.5	0	1	2.38	6.23	0.0129	194.45	0.030	10.18	116.93	8.61
15	5.5	0.5	50	3	2.86	NG	0.0000		0.000			
15	5.5	2.5	0	3	2.62	NG	0.0000		0.000			
19	7.5	0.5	0	3	3.01	6.04	0.0443	27.05	0.098	3.06	4.43	9.05
19	7.5	0.5	100	3	3.01	5.52	0.0488	24.49	0.099	3.04	3.94	8.53
19	7.5	0.5	200	3	2.92	5.94	0.0382	35.78	0.082	3.71	8.68	8.86
19	7.5	0.5	1000	3	2.93	5.49	0.0278	45.42	0.056	5.67	7.95	8.43
19	7.5	2.5	0	3	2.99	4.43	0.0785	23.78	0.128	2.35	11.02	7.43
19	7.5	2.5	100	3	2.93	4.51	0.0738	25.70	0.119	2.65	10.62	7.43
19	7.5	2.5	200	3	2.98	5.20	0.0475	34.89	0.086	3.69	10.58	8.18
19	7.5	2.5	1000	3	2.92	2.98	0.0559	29.43	0.050	6.45	5.59	5.90
19	7.5	4.0	0	5	2.54	5.24	0.0187	197.99	0.037	9.18	141.11	7.78
19	7.5	4.0	50	2	2.37	5.07	0.0229	133.84	0.043	7.19	90.07	7.42
19	7.5	4.0	100	3	2.51	NG	0.0000		0.000			

19	7.5	4.0	200	3	2.50	NG	0.0000		0.000			
19	7.5	4.0	1000	3	2.53	NG	0.0000		0.000			
19	6.5	0.5	0	5	2.86	5.79	0.0472	25.40	0.101	3.04	3.98	8.65
19	6.5	0.5	50	1	2.95	5.93	0.0268	38.17	0.058	5.15	0.86	8.88
19	6.5	0.5	100	5	2.85	5.77	0.0367	37.44	0.077	4.37	6.37	8.62
19	6.5	0.5	200	4	2.66	5.43	0.0312	41.40	0.062	5.16	7.41	8.09
19	6.5	0.5	1000	3	2.61	NG	0.000		0.000			
19	6.5	2.5	0	8	2.55	5.09	0.0225	124.58	0.042	8.35	74.63	7.63
19	6.5	2.5	50	2	2.99	5.31	0.0427	63.34	0.085	3.58	40.15	8.30
19	6.5	2.5	100	5	2.57	NG	0.0000		0.000			
19	6.5	2.5	200	1	2.02	NG	0.0000		0.000			
19	6.5	4.0	0	3	3.16	4.38	0.0198	178.49	0.032	9.47	127.87	7.54
19	6.5	4.0	50	3	3.03	NG	0.0000		0.000			
19	6.5	4.0	100	3	3.01	NG	0.0000		0.000			
19	6.0	0.5	0	2	3.32	3.98	0.0426	162.25	0.063	4.87	138.62	7.30
19	6.0	0.5	50	2	2.67	NG	0.0000		0.000			
19	6.0	0.5	100	2	2.61	NG	0.0000		0.000			
19	6.0	2.5	0	3	3.02	5.93	0.0353	84.49	0.077	3.94	55.87	8.95
19	6.0	2.5	50	2	2.85	NG	0.0000		0.000			
19	6.0	2.5	100	2	2.83	NG	0.0000		0.000			
19	5.5	0.5	0	5	2.86	5.93	0.0380	90.85	0.083	3.67	64.32	8.79
19	5.5	0.5	50	3	2.99	NG	0.0000		0.000			
19	5.5	2.5	0	2	2.56	5.18	0.0450	194.88	0.084	3.95	169.57	7.73
19	5.5	2.5	50	3	2.35	NG	0.0000		0.000			
19	5.5	4.0	0	3	2.79	NG	0.0000		0.000			
28	7.5	0.5	0	3	2.71	6.12	0.1808	7.19	0.407	0.74	1.65	8.83
28	7.5	0.5	200	3	2.81	5.97	0.1890	7.38	0.415	0.72	2.09	8.78
28	7.5	2.5	0	3	3.00	5.88	0.1751	11.22	0.379	0.80	5.51	8.88
28	7.5	2.5	200	3	2.82	5.83	0.1662	10.72	0.357	0.85	4.69	8.66
28	7.5	4.0	0	6	2.95	5.71	0.0871	20.72	0.170	1.83	7.65	8.66
28	7.5	4.0	100	6	2.98	4.99	0.1166	19.24	0.212	1.47	10.23	7.97
28	7.5	4.0	200	3	2.90	4.80	0.0839	18.02	0.148	2.05	5.81	7.70
28	7.0	0.5	0	3	2.88	5.77	0.1996	7.56	0.418	0.72	2.48	8.66

Table 2 (continued)

Temperature	pH	NaCl (%)	NaNO ₂ (ppm)	REP	Gompertz parameters				EGR (Log/h)	GT (h)	LPD (h)	MPD (Log)
					A	C	B	M				
28	7.0	0.5	200	3	2.80	6.02	0.1817	8.04	0.403	0.75	2.53	8.83
28	7.0	2.5	0	2	2.87	6.27	0.1656	9.72	0.382	0.79	3.67	9.14
28	7.0	2.5	200	2	2.84	6.12	0.1614	9.96	0.363	0.83	3.76	8.96
28	7.0	2.5	1000	2	2.93	5.44	0.1729	11.69	0.346	0.87	5.91	8.37
28	7.0	4.0	0	2	2.61	6.69	0.0975	16.10	0.240	1.26	5.77	9.30
28	7.0	4.0	200	2	2.68	6.49	0.0808	17.99	0.193	1.57	5.60	9.16
28	7.0	4.0	1000	2	3.04	5.13	0.1061	27.64	0.200	1.52	18.15	8.17
28	6.5	0.5	0	2	2.07	6.81	0.1702	8.62	0.426	0.71	2.74	8.87
28	6.5	0.5	100	2	2.15	6.27	0.1596	9.66	0.368	0.82	3.38	8.42
28	6.5	0.5	200	2	2.05	6.37	0.0920	16.41	0.216	1.47	4.99	8.42
28	6.5	0.5	1000	3	2.42	NG	0.0000		0.000			
28	6.5	2.5	0	4	2.59	5.92	0.2178	8.30	0.473	0.65	3.62	8.51
28	6.5	2.5	100	4	2.69	5.58	0.1868	9.40	0.391	0.78	4.04	8.38
28	6.5	2.5	200	4	2.63	5.69	0.1193	14.51	0.244	1.48	3.97	8.32
28	6.5	2.5	1000	2	2.61	NG	0.0000		0.000			
28	6.5	4.0	0	2	3.02	4.86	0.1732	16.19	0.312	1.01	10.31	7.88
28	6.5	4.0	100	1	3.01	4.95	0.2673	22.03	0.487	0.62	18.29	7.96
28	6.5	4.0	200	1	2.97	3.54	0.1013	12.79	0.132	2.28	2.92	6.51
28	6.5	4.0	1000	3	3.04	NG	0.0000		0.000			
28	6.0	0.5	0	2	2.79	5.69	0.1930	7.15	0.419	0.75	1.96	8.47
28	6.0	0.5	50	2	2.59	5.66	0.1682	7.28	0.350	0.87	1.30	8.25
28	6.0	0.5	100	1	3.38	5.97	0.1115	31.3	0.245	1.23	22.33	9.35
28	6.0	2.5	0	2	2.81	5.67	0.1975	10.05	0.412	0.74	4.99	8.48
28	6.0	2.5	50	2	2.71	4.94	0.0692	18.17	0.126	2.44	3.54	7.64
28	6.0	2.5	100	1	3.01	4.44	0.0554	207.6	0.090	3.33	189.59	7.54
28	6.0	2.5	200	2	2.75	NG	0.0000		0.000			
28	6.0	4.0	0	3	2.76	5.16	0.1457	12.77	0.270	1.14	5.45	7.92
28	6.0	4.0	50	4	2.81	5.25	0.0640	53.42	0.124	2.97	34.50	8.06
28	6.0	4.0	100	2	2.56	5.62	0.0220	181.80	0.046	6.82	135.17	8.18
28	5.5	0.5	0	3	3.10	5.27	0.1930	12.25	0.372	0.81	7.04	8.38

28	5.5	0.5	50	3	3.12	NG	0.0000		0.000				
28	5.5	0.5	100	3	3.07	NG	0.0000		0.000				
28	5.5	0.5	200	3	3.03	NG	0.0000	0.000					
28	5.5	2.5	0	3	3.06	4.18	0.1635	15.77	0.250	1.21	9.57	7.24	
28	5.5	2.5	50	6	3.01	NG	0.0000		0.000				
28	5.5	2.5	100	3	2.97	NG	0.0000		0.000				
28	5.5	4.0	0	3	2.86	4.81	0.0335	37.30	0.059	5.19	7.15	7.67	
28	5.5	4.0	50	3	2.96	NG	0.0000		0.000				
28	5.5	4.0	100	3	2.96	NG	0.0000		0.000				
37	7.5	0.5	0	3	2.97	5.96	0.3878	4.45	0.850	0.35	1.87	8.92	
37	7.5	0.5	200	3	2.92	6.12	0.3750	4.68	0.843	0.36	2.00	9.03	
37	6.5	0.5	0	3	3.06	5.57	0.4026	4.61	0.824	0.37	2.12	8.63	
37	6.5	0.5	200	3	3.00	5.49	0.3691	5.05	0.745	0.41	2.33	8.49	
37	6.5	2.5	0	3	2.91	5.57	0.3679	5.63	0.752	0.40	2.89	8.48	
37	6.5	2.5	200	3	2.87	5.23	0.3099	5.80	0.594	0.52	2.48	8.10	
37	6.5	4.0	0	3	3.18	4.55	0.2421	10.06	0.408	0.76	5.88	7.73	
37	6.5	4.0	100	3	3.25	4.17	0.2217	16.62	0.352	1.03	11.82	7.42	
37	6.5	4.0	200	3	3.18	NG	0.0000		0.000				
37	6.5	4.0	1000	2	3.17	NG	0.0000		0.000				
37	6.0	2.5	0	2	3.04	4.87	0.3846	4.96	0.693	0.45	2.31	7.91	
37	6.0	2.5	100	2	2.91	3.30	0.3781	6.38	0.329	0.92	2.51	6.21	
37	6.0	4.0	0	2	2.91	4.62	0.3111	5.76	0.529	0.57	2.54	7.53	
37	6.0	4.0	50	2	2.85	4.37	0.1753	8.04	0.282	1.09	2.18	7.21	
37	5.5	0.5	0	3	2.99	4.97	0.2758	6.50	0.503	0.60	2.86	7.96	
37	5.5	0.5	50	2	3.09	5.67	0.1022	22.78	0.213	1.43	12.88	8.75	
37	5.5	2.5	0	3	2.99	4.96	0.2634	6.42	0.480	0.63	2.60	7.95	
37	5.5	2.5	50	2	3.00	5.24	0.2577	12.07	0.494	0.61	8.17	8.24	
37	5.5	2.5	100	1	3.09	5.08	0.1025	29.47	0.192	1.57	19.71	8.17	
37	5.5	2.5	200	3	3.13	NG	0.0000		0.000				
37	5.5	4.0	0	3	2.83	4.94	0.1929	9.47	0.348	0.87	4.27	7.78	
37	5.5	4.0	50	3	2.21	NG	0.0000		0.000				
37	5.5	4.0	100	3	2.37	NG	0.0000		0.000				

NG, no growth.

Statistical analysis. Second-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, 1989, pp. 871–996). The regression analysis was performed on the untransformed Gompertz parameters B and M and on several transformations of the parameters including $\text{Ln}(M)$ and $\text{Ln}(B)$ (excluding no-growth data), $\text{Sqrt}(B)$ and $\text{Sqrt}(1/M)$ (all data), $\text{Ln}(B + 0.0001)$, $\text{Ln}(1/M + 0.0001)$ (all data), and $\text{Ln}(GT)$ and $\text{Ln}(LPD)$ (excluding no-growth data). Third-order response surface model was also calculated and regression analysis was performed on the Ln transformations. Second and third-order models for the Ln transformations were also submitted to stepwise regression using backward elimination model selection technique (Draper and Smith, 1981, pp. 305–307).

3. Results and discussion

Data from 375 cultures, representing 124 variable combinations, were used to derive the models to predict the anaerobic growth of *S. flexneri* as a function of temperature, sodium chloride and sodium nitrite concentrations and initial pH. Growth curves were generated from the bacterial population data using the Gompertz function (Table 1) and exponential growth rates, generation times, lag times and maximum population densities were calculated. This function has been used extensively in our laboratory to describe the growth of a variety of bacteria. Zwietering et al. (1990) compared several sigmoidal functions to describe the bacterial growth curve and concluded that the Gompertz equation was statistically sufficient to describe the growth data of *Lactobacillus plantarum*, as well as other bacteria, and was easy to use. The growth kinetics data for each variable combination are summarized in Table 2. Growth of *S. flexneri* was not observed under the conditions corresponding to 40 of the variable combinations studied. An additional 15 variable combinations resulted in environments under which some of the replicate cultures grew, while others did not. These are listed in Table 3. *S. flexneri* grew in 233 of the total of 375 cultures, but failed to grow or died off in 142 cultures. The data obtained indicate that the variables studied (temperature, sodium chloride and sodium nitrite concentrations, initial pH) interact to affect the anaerobic growth of the organism. Antimicrobial activity of sodium nitrite toward *S. flexneri* increased with decreasing temperature, decreasing pH and increasing sodium chloride concentration. The organism did not grow at 12°C. It grew at 15°C at all pH values studied in the absence of sodium nitrite, but failed to grow at pH 5.5–6.5 in the presence of 50 ppm NaNO_2 , the lowest concentration tested. Increasing concentrations of sodium chloride progressively inhibited bacterial growth, particularly at low temperature. In the absence of sodium nitrite the microorganism grew in media containing 4% NaCl at 19–37°C except at 19°C in combination with a pH of 5.5. Overall, the results were similar to those obtained previously for growth of the organism under aerobic conditions (Zaika et al., 1989, 1991). However, under anaerobic conditions the maximum population densities

Table 3

Conditions of temperature (T), pH (P), sodium chloride (S) and sodium nitrite (N) concentrations that resulted in variable growth (growth, no growth) of *S. flexneri*

T	P	S	N	Total cultures	No growth
15	5.5	0.5	0	3	2
19	5.5	0.5	0	6	1
19	5.5	2.5	0	7	5
19	6.5	2.5	0	9	1
19	6.5	2.5	50	8	6
19	7.5	4.0	0	6	1
19	7.5	4.0	50	3	1
28	6.0	0.5	100	2	1
28	6.0	2.5	100	2	1
28	6.0	4.0	50	5	1
28	6.0	4.0	100	3	1
28	6.5	4.0	200	2	1
37	5.5	0.5	50	3	1
37	5.5	2.5	50	5	3
37	5.5	2.5	100	5	4

(mean value, 8.20 cfu/ml) were generally lower than those obtained under aerobic conditions (mean value, 9.13 cfu/ml; Zaika et al., 1992).

Previously we developed models for aerobic growth of *S. flexneri* (Zaika et al., 1992) in terms of the Gompertz B and M parameters. The Gompertz A and C values were omitted from consideration in model development since the growth kinetics were not affected by initial inoculum size and the maximum population densities (MPD) attained were independent of the variables unless two or more factors were approaching limiting values. This approach was also used by Gibson et al. (1988), Buchanan and Phillips (1990), Palumbo et al. (1991), Buchanan et al. (1993) in the development of models for the growth of *Salmonella*, *Listeria monocytogenes*, *Aeromonas hydrophila*, and *Escherichia coli* 0157:H7, respectively.

Supplemental analyses were carried out to determine if these assumptions were valid for the anaerobic growth of *S. flexneri*. The effect of four inoculum levels on the growth kinetics of *S. flexneri* was examined for the experimental parameter combination of 28°C, pH 5.5, 2.5% sodium chloride and 0 ppm sodium nitrite (Table 4). The inoculum level (Gompertz A value) affected the Gompertz C , B and M values. However, regression analysis indicated that these terms varied in such a manner that the inoculum size had no significant effect on the derived values for exponential growth rate (EGR), generation time (GT) and lag phase duration (LPD). The data suggest that the growth kinetics are not substantially affected by the size of the initial inoculum. It was also observed that for the majority of variable combinations, if the organism initiated growth, it achieved a MPD of approximately 10^8 cfu/ml (Table 2). On this basis the Gompertz A and C values were omitted from consideration for model development.

A total of seven second-order models and four third-order models were generated using various transformations to stabilize the variance and assess possible

Table 4

Effect of inoculum size on growth kinetics ^a of *S. flexneri* in BHI adjusted to pH 5.5, containing 2.5% (w/v) NaCl and 0 ppm NaNO₂, at 28°C

	<i>A</i>	<i>C</i>	<i>B</i>	<i>M</i>	EGR	GT	LPD	MPD
Mean	2.00	5.73	0.1285	14.89	0.271	1.11	7.11	7.73
SD (<i>n</i> = 3)	(0.06)	(0.13)	(0.0030)	(0.26)	(0.001)	(0.01)	(0.30)	(0.15)
Mean	2.92	4.96	0.1560	12.39	0.285	1.06	5.97	7.89
SD (<i>n</i> = 3)	(0.01)	(0.14)	(0.0064)	(0.28)	(0.006)	(0.03)	(0.09)	(0.12)
Mean	4.04	3.45	0.2081	11.02	0.263	1.14	6.21	7.49
SD (<i>n</i> = 3)	(0.01)	(0.23)	(0.0100)	(0.21)	(0.008)	(0.04)	(0.12)	(0.23)
Mean	4.97	2.20	0.3450	8.29	0.280	1.08	5.39	7.18
SD (<i>n</i> = 3)	(0.02)	(0.06)	(0.0137)	(0.04)	(0.017)	(0.06)	(0.15)	(0.05)
All data								
Mean					0.274	1.10	6.16	7.57
SD (<i>n</i> = 12)					(0.012)	(0.05)	(0.67)	(0.31)

^a Abbreviations as in Table 1; SD, standard deviation; *n*, number of replicate cultures.

Table 5

Comparison of the goodness of fit ^a of response surface models for anaerobic growth of *S. flexneri*

		<i>R</i> ²	Adj. <i>R</i> ²	<i>n</i> ^b
Second-order models				
Model 1	<i>B</i>	0.743	0.796	375
	<i>M</i>	0.613	0.676	233
Model 2	Ln(<i>B</i>)	0.862	0.896	233
	Ln(<i>M</i>)	0.836	0.870	233
Model 3	Sqrt(<i>B</i>)	0.718	0.775	375
	Sqrt(1/ <i>M</i>)	0.743	0.781	375
Model 4	Ln(<i>B</i> + 0.0001)	0.616	0.692	375
	Ln[(1/ <i>M</i>) + 0.0001]	0.643	0.710	375
Model 5	Ln(LPD)	0.873	0.899	233
	Ln(LPD)	0.714	0.765	233
Model 6	Ln(<i>B</i>) after SRBE ^c	0.858	0.892	233
	Ln(<i>M</i>) after SRBE	0.834	0.868	233
Model 7	Ln(GT) after SRBE	0.871	0.897	233
	Ln(LPD) after SRBE	0.712	0.762	233
Third-order models				
Model 8	Ln(<i>B</i>)	0.906	0.942	233
	Ln(<i>M</i>)	0.896	0.933	233
Model 9	Ln(GT)	0.917	0.944	233
	Ln(LPD)	0.807	0.864	233
Model 10	Ln(<i>B</i>) after SRBE	0.904	0.940	233
	Ln(<i>M</i>) after SRBE	0.893	0.929	233
Model 11	Ln(GT) after SRBE	0.915	0.942	233
	Ln(LPD) after SRBE	0.795	0.851	233

^a Adjusted $R^2 = R^2 / \text{Max } R^2$ (Draper and Smith, 1981, pp. 40–42).

^b No. of observations; no-growth responses were treated as missing values.

^c Stepwise Regression-Backward Elimination Method (with $\alpha = 0.10$).

approaches to the inclusion of no-growth data (Table 5). This included second and third-order models of the $\text{Ln}(B)$ and $\text{Ln}(M)$, as well as $\text{Ln}(GT)$ and $\text{Ln}(LPD)$, submitted to stepwise regression using backward elimination method to remove non-significant terms. Examination of the R^2 values (Table 5) indicated that the Ln transformations gave the best fits, with the third-order models providing a

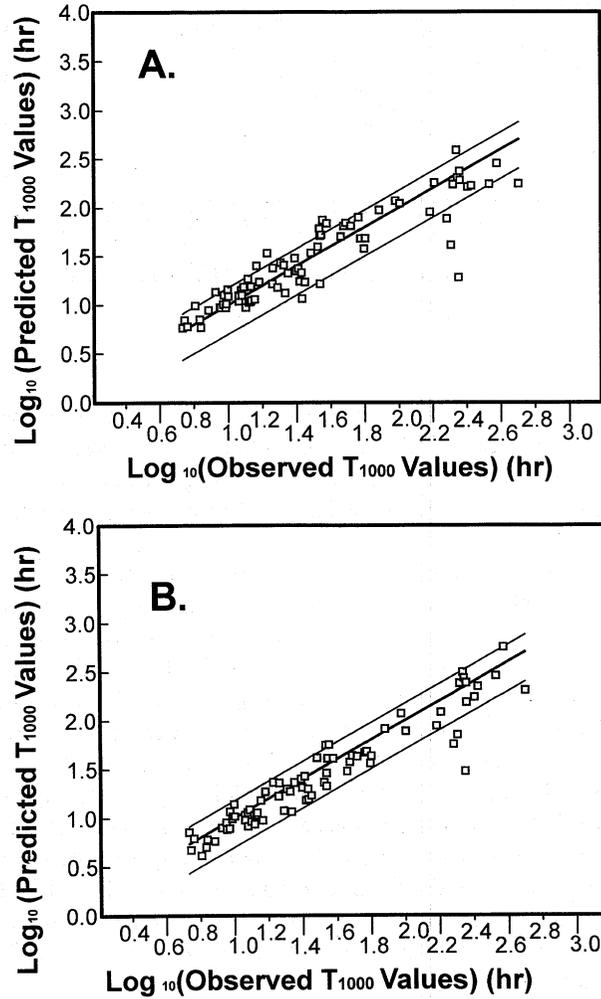


Fig. 1. Comparison of observed and predicted T_{1000} values (time to 1000-fold increase in population density) for (A) Model 2 (Full Regression/Second-order/Gompertz terms), (B) Model 10 (Stepwise Regression-Backward Elimination/Third-order/Gompertz terms), and (C) Model 7 (Stepwise Regression-Backward Elimination/Second-order/Kinetics terms). T_{1000} values were calculated for Model 2 and Model 10 using the equation $T_{1000} = (0.54412/B) + M$, and for Model 7 using $T_{1000} = (10.1155 \text{ GT}) + \text{LPD}$. Predictions for variable combinations corresponding to no-growth observation are not included in the figures. The center line represents the line of identity and the two flanking lines are $\pm 50\%$ of the observed value.

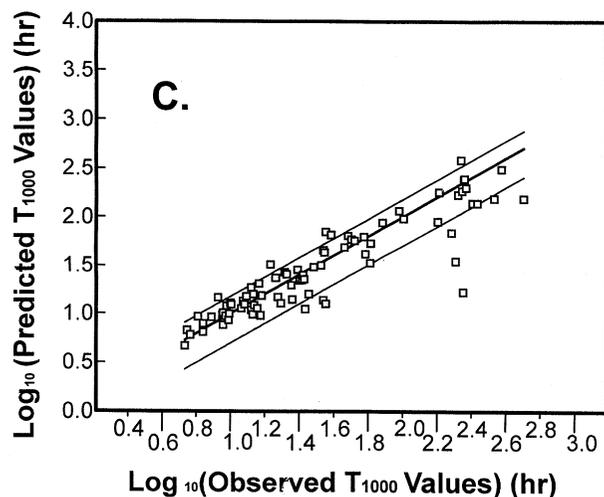


Fig 1 (continued).

substantial increase in R^2 values. The Ln(GT) models had R^2 values similar to those for the Ln(B) and Ln(M) models, while the R^2 values for the Ln(LPD) models indicated poorer fit. R^2 values were decreased to only a very small degree when the Ln-based models were submitted to stepwise regression-backward elimination procedure.

The Ln transformation based models were then evaluated by comparison of predicted versus observed values. Gompertz parameters of $A = 2.84$ and $C = 5.36$, the grand means of the experimental data, were used in conjunction with the terms predicted by the models to calculate predicted GT, LPD and T_{1000} (time to 1000-fold increase in population density) values. The best overall fit for T_{1000} values observed with a full regression model was with Model 2, the second-order model for the Ln(B) and Ln(M) terms (Fig. 1A, Table 6). The best stepwise regression-backward elimination based model in regard to agreement between observed and predicted values for variable combinations that supported growth was Model 10, the third-order model for Ln(B) and Ln(M) (Fig. 1B, Table 6). However, almost equally good agreement was observed with Model 7, the second-order model for Ln(GT) and Ln(LPD) (Fig. 1C, Table 6). It should be noted that the applicable temperature range for these models is 15–37°C, since the Ln transformation excluded the no-growth data at 12°C.

To our knowledge, there are virtually no published data on the growth kinetics of *Shigella* that we could use to evaluate the effectiveness of the growth models obtained. Recently Islam et al. (1993) reported that *S. flexneri* grew readily in a number of foods (boiled rice, lentil soup, milk, cooked beef, cooked fish, mashed potato, mashed eggplant, raw cucumber) at 37° and 25°C. However, the data presented did not permit accurate calculation of growth kinetics parameters for comparison with corresponding values estimated with the models.

Table 6

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 anaerobic growth of *Shigella flexneri*

Model 2 Full Regression Second-order models of Gompertz B and M values

$$\begin{aligned} \text{Ln}(B) = & -12.5517 + 0.3588T + 1.0654P + 0.00239S - 0.00709N + 0.00784TP \\ & + 0.00498TS - 0.0000178TN + 0.0413PS + 0.00089PN + 0.000106SN - 0.0051T^2 \\ & - 0.0883P^2 - 0.1346S^2 + 0.00000059N^2 \end{aligned}$$

$$\begin{aligned} \text{Ln}(M) = & 26.9414 - 0.6728T - 3.8771P + 0.1625S + 0.0115N + 0.0113TP - 0.013TS \\ & + 0.000078TN - 0.00625PS - 0.00179PN - 0.000256SN + 0.00851T^2 + 0.2473P^2 \\ & + 0.1217S^2 + 0.00000041N^2 \end{aligned}$$

Model 10 Stepwise Regression-Backward Elimination Third-order models of Gompertz B and M values

$$\begin{aligned} \text{Ln}(B) = & -68.3261 - 0.8526T + 33.9897P - 9.2741S - 0.2673N + 0.3421TP + 0.1213TS \\ & - 0.00091TN + 2.4031PS + 0.0789PN - 0.00536SN - 6.1274P^2 - 0.1279S^2 \\ & - 0.00105T^2P + 0.0000174T^2N + 0.348P^3 - 0.0204TP^2 - 0.1444P^2S - 0.00554P^2N \\ & + 0.00000001N^3 - 0.00000184PN^2 - 0.0177TPS + 0.000765PSN \end{aligned}$$

$$\begin{aligned} \text{Ln}(M) = & 96.178 - 40.8424P + 11.3887S + 0.3755N - 0.3498TP - 0.2726TS + 0.00142TN \\ & - 2.354PS - 0.1131PN + 0.01SN + 0.0312T^2 + 6.8897P^2 - 0.000548T^3 \\ & + 0.00326T^2P + 0.00171T^2S - 0.0000267T^2N - 0.3668P^3 + 0.0133TP^2 \\ & + 0.1263P^2S + 0.00811P^2N + 0.019PS^2 + 0.0248TPS - 0.00142PSN \end{aligned}$$

Model 7 Stepwise Regression-Backward Elimination Second-order models of growth kinetics values for GT and LPD

$$\begin{aligned} \text{Ln}(GT) = & 8.0275 - 0.4056T + 0.00144N - 0.00856TP - 0.00581TS - 0.0272PS + 0.0061T^2 \\ & + 0.1272S^2 - 0.00000105N^2 \end{aligned}$$

$$\begin{aligned} \text{Ln}(LPD) = & 35.5866 - 0.9291T - 5.6129P + 0.0214TP - 0.0202TS + 0.000286TN \\ & + 0.1013PS - 0.00104PN - 0.00065SN + 0.0121T^2 + 0.3345P^2 + 0.0735S^2 \\ & + 0.00000197N^2 \end{aligned}$$

We have initiated studies on the growth of *S. flexneri* in a variety of foods to obtain a fuller evaluation of the growth models. Undoubtedly, there are additional factors that influence the growth of *S. flexneri* and may play an important role in particular types of food. These may need to be taken into consideration to refine the growth models. However, based on comparisons of R^2 values (Table 5), residuals (not shown), comparisons of predicted versus observed values, and lack of anomalous predictions, we consider that Model 10 presented here can be useful to persons involved in the manufacture, storage or handling of food to estimate the likelihood that *Shigella*, if present, would grow under a given set of conditions. A computer-based pathogen modelling program has been developed in our laboratory that makes available, in a user-friendly manner, growth models for a number of foodborne bacteria. Model 10, describing the anaerobic growth of *S. flexneri*, will be incorporated into the newest version of this program.

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