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Growth of *Shigella flexneri* in foods: comparison of observed and predicted growth kinetics parameters¹

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Abstract

Shigella causes foodborne gastrointestinal illness; however, little information is available on its ability to grow in foods. Commercially available sterile foods (UHT milk, beef broth, chicken broth, vegetable broth, meats, vegetables) were inoculated with *S. flexneri* 5348 and incubated at 12, 15, 19, 28 or 37°C. Growth curves were fitted from plate count data by the Gompertz equation and exponential growth rates, generation times, lag times and maximum population densities were derived. The observed kinetics values, expressed as T₁₀₀₀ (time, h, required for a 3 log increase in bacterial population), were compared with values calculated using published growth models. Observed and calculated values compared favorably for growth at 19–37°C. *S. flexneri* grew well in milk at 15–37°C but growth at 12°C was variable. The bacteria readily grew in most foods, even at 12°C; but died off in carrots at 19 and 28°C. Factors other than those used in the growth model may influence bacterial growth in specific foods.

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

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1. Introduction

Shigella is a major cause of foodborne gastrointestinal illness (Smith, 1987). Its habitat is the intestinal tract of humans and other primates. Thus, the source of *Shigella* to food is waters polluted with fecal material and infected food handlers. All four species of the genus *Shigella* — *S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei* — are capable of causing gastrointestinal illness. Data on foodborne outbreaks reported to the Centers for Disease Control for the years 1973–1987 indicated that *Shigella* species were identified in 104 outbreaks (14 399 cases) out of 1869 outbreaks (108 906 cases) of known etiology involving bacteria. Much higher estimates of the number of foodborne shigellosis cases per year in the United States have been proposed (Todd, 1989; Archer and Kvenberg, 1985). Epidemiological data indicate that poor personal hygiene is the most frequently implicated contributing factor in foodborne shigellosis outbreaks (Bean and Griffin, 1990).

Foods that have been associated with shigellosis outbreaks are (1) foods that are consumed raw, (2) multiple ingredient foods and (3) cooked foods that are not reheated before serving. Recent shigellosis outbreaks have been associated with commercially shredded lettuce (Davis et al., 1988), German potato salad (Lew et al., 1991) and uncooked tofu salad (Lee et al., 1991). Since the infectious dose of *Shigella* is very low, 10–500 organisms (DuPont et al., 1989), the potential for gastrointestinal illness due to consumption of foods contaminated with *Shigella* is relatively high. Studies of *Shigella* spp. in foods are limited; however, it appears that these organisms are capable of surviving for extended periods of time in different foods under various conditions (Siegmond, 1960; Taylor and Nakamura, 1964). Satchell et al. (1990) demonstrated that *S. sonnei* can survive and even proliferate in shredded cabbage. *S. sonnei*, *S. flexneri* and *S. dysenteriae* can grow in sliced papaya and jicama at room temperature (Fernandez Escartin et al., 1989). 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°C and 25°C. Although much attention has been given to investigations of the taxonomy, epidemiology and virulence of *Shigella* spp., few systematic studies dealing with the growth characteristics of the organism have been reported (Fehlhaber, 1981).

To control the proliferation of a pathogenic microorganism in food, it is necessary to understand its growth characteristics. There is an increasing interest in microbial growth models, based on growth kinetics data, which are useful in obtaining 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 describing the combined effects 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). The growth kinetics parameters used to develop the models have been published (Zaika et al., 1989,

1991, 1994). The models for the growth of *S. flexneri* have been incorporated into the computerized Pathogen Modeling Program Version 4.0, available from our laboratory. The objective of the present work was to compare the growth kinetics values for *S. flexneri* inoculated into foods with values calculated using the growth models on the basis of the same conditions of temperature, pH, sodium chloride and sodium nitrite levels.

2. Materials and methods

2.1. Microorganism

Shigella flexneri 5348 (obtained from Dr. David W. Niesel, University of Texas Medical Branch, Galveston, TX) was used throughout the study. The same strain had been used previously to develop the growth models. 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.

2.2. Foods

Commercially available sterile foods that did not require further handling were used throughout the study. These were: UHT milk, canned beef broth, canned chicken broth, canned vegetable broth, baby foods (chicken and broth, carrots, peas, squash), canned dogfood (beef), canned pink salmon and canned lunchmeat. The sodium chloride concentrations were calculated from the levels of sodium listed on the labels. Determination of the pH values of the liquid foods was carried out by direct measurement. Determination of the pH values of semisolid foods was done using slurries prepared from either 1 part baby food + 4 parts distilled water or 1 part canned food + 9 parts distilled water.

2.3. Growth of *S. flexneri* in foods

Liquid foods, 100 ml, were aseptically transferred into 250-ml sterile Erlenmeyer flasks and inoculated with *S. flexneri* to a level of 10^3 CFU/ml. Semisolid foods, 100 g, were aseptically transferred to sterile 300-ml beakers and inoculated with *S. flexneri* to a level of 10^4 CFU/g. Triplicate samples of all foods were prepared. The containers were capped with sterile aluminium foil and placed on incubator shelves at the desired temperatures. This will be referred to as 'stationary' growth.

For aerobic growth studies, 50-ml portions of UHT milk were aseptically transferred to sterile 250-ml Erlenmeyer flasks and inoculated to an initial level of 10^3 CFU/ml. The flasks were capped with sterile foam plugs and incubated on a rotary shaker (150 rev./min) at the desired temperature. Anaerobic growth of *S.*

flexneri in UHT milk was studied using the procedure described previously (Zaika et al., 1994).

2.4. Determination of microbial populations in the foods

Immediately after inoculation and after suitable periods of incubation, portions of foods were aseptically withdrawn from the containers and dilutions in sterile 0.1% peptone water prepared as necessary. The bacterial suspensions were surface plated on Tryptic Soy Agar (Difco) using a Spiral Plater (Spiral System Instruments Inc., Bethesda, MD). Liquid foods were plated directly or as appropriate dilutions in peptone water. For semisolid foods, 1 g of sample was weighed into a test tube containing 9 ml of peptone water and mixed thoroughly by vortexing. The mixture was poured into a filter stomacher bag (SFB-0410, Spiral Biotech., Bethesda, MD) to remove particulate matter. The filtrate was plated directly or diluted with peptone water. The plates (in duplicate) were incubated for 24 h at 37°C and counted with the aid of a Bacteria Colony Counter Model 500A (Spiral System Instruments Inc., Bethesda, MD). Portions of foods serving as the uninoculated controls were subjected to the same conditions of incubation and analysis as those inoculated with *S. flexneri*. The uninoculated controls were free from microbial contaminants.

2.5. Curve fitting

Growth curves were generated from the experimental growth data using the Gompertz equation (Table 1) in conjunction with ABACUS, a non-linear regression program that employs a Gauss-Newton iteration procedure. This FORTRAN-

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
= $\{-\ln [-\ln (3/C)]\}/B + M$

based program was developed by W.C. Damert (US Department of Agriculture, Eastern Regional Research Center, 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 ((log₁₀ CFU/ml)/h), generation times (h) and maximum population densities (log₁₀ CFU/ml) as described by Gibson et al. (1987) (Table 1).

3. Results and discussion

To determine how well the growth of *S. flexneri* in foods can be estimated by growth models, we studied the growth of the bacterium in a variety of foods in the temperature range defined by the growth models. Foods chosen for the study were those that were sterile, homogeneous and could be used with a minimum of handling. Triplicate portions of each food were inoculated with *S. flexneri* and incubated at selected temperatures (12, 15, 19, 28, 37°C).

Gompertz equation parameters and the derived growth kinetics values for *S. flexneri* cultured in foods are shown in Table 2. The bacterium grew readily in most foods even at 12 and 15°C. Three brands of canned beef broth were investigated. Brands B and C were from the same manufacturer with brand B being a reduced salt product. *Shigella* grew readily at 12°C in beef broth A, but failed to grow in beef broth B. Differences in the formulation in the same type of product, besides salt levels, undoubtedly have an influence on the ability of bacteria to grow in the product. *S. flexneri* grew consistently faster in beef broth A than broths B or C. The organism grew readily in canned chicken broth at 19°C. Growth did not occur in canned vegetable broth at 12°C and was slow at 19°C.

Several baby foods were inoculated with *S. flexneri* to assess its potential for growth in a single-ingredient food product. The organism grew well in chicken with broth, peas and squash even at 15°C. However, no growth was obtained in carrots at 19 or 28°C. The bacterial population decreased to undetectable levels in 55 h at 28°C, more slowly at 19°C. Although the carrot product had a pH of 5.05, the lowest of the products tested, pH does not appear to be the limiting factor since *S. flexneri* grew readily at 28°C in Brain-Heart Infusion broth of pH 4.75 (data not shown). Babic et al. (1994) reported that ethanolic extracts of peeled and shredded carrots had antimicrobial activity against foodborne bacteria and yeast. Beuchat and Brackett (1990) reported that raw carrots were toxic to *Listeria monocytogenes*, although the antilisterial effect was essentially eliminated when the carrots were cooked.

S. flexneri grew well in canned dogfood (ground beef) and in canned pink salmon in the temperature range studied (12–28°C). Good growth was obtained in canned lunchmeat A at 28°C. Growth did not occur in lunchmeat B at 19°C, and a one log decrease in bacterial population was obtained after 270 h of incubation.

Growth of *S. flexneri* in UHT milk was studied at 28, 19, 15 and 12°C under three different conditions: (1) aerobic/shaken, (2) anaerobic/shaken, container flushed with nitrogen, (3) 'stationary' (access to air, but not shaken). The Gompertz

Table 2
Gompertz equation parameters and growth kinetics values for *Shigella flexneri* cultured in foods

Food	Temperature (°C)	pH	NaCl (%)	A	C	B	M	EGR [(log CFU/ml)/h]	GT (h)	LPD (h)	MPD (log CFU/ml)
Canned broths											
Beef broth A	37	5.90	0.9	3.02	4.53	0.5605	3.81	0.931	0.33	1.99	7.55
Beef broth B	37	6.05	0.7	2.65	5.28	0.4076	6.55	0.792	0.38	4.09	7.93
Beef broth C	37	6.10	1.4	3.15	5.17	0.3808	6.20	0.725	0.42	3.56	8.32
Beef broth A	19	5.90	0.9	3.19	5.13	0.0778	21.49	0.146	2.07	8.50	8.32
Beef broth B	19	6.05	0.7	2.69	5.61	0.0467	52.36	0.096	3.13	30.93	8.32
Beef broth C	19	6.10	1.4	2.69	5.95	0.0300	68.05	0.067	4.51	32.25	8.64
Beef broth A	12	5.90	0.9	3.08	5.68	0.0231	72.93	0.048	6.29	32.38	8.75
Beef broth B	12	6.05	0.7	3.06	NG	0.0000					
Chicken broth	19	6.60	0.7	3.20	6.28	0.0480	32.45	0.111	2.71	11.60	9.48
Veg. broth	37	5.48	1.1	2.90	4.58	0.2381	9.08	0.400	0.75	4.86	7.48
Veg. broth	19	5.48	1.1	3.81	5.38	0.0245	209.91	0.048	6.28	168.49	8.56
Veg. broth	12	5.48	1.1	3.39	NG	0.0000					
Baby foods											
Carrots	28	5.05	0.2	3.74	NG	0.0000					
Carrots	19	5.05	0.2	4.08	NG	0.0000					
Peas	15	6.51	0.0	2.68	4.58	0.0467	35.96	0.079	3.98	13.95	7.25
Squash	28	5.37	0.0	3.34	3.72	0.2208	7.81	0.302	1.00	3.27	7.06
Squash	15	5.37	0.0	3.33	3.01	0.0308	45.87	0.033	9.47	12.44	6.24
Chicken + broth	37	6.43	0.2	4.34	4.20	0.5535	4.27	0.840	0.35	2.46	8.54
Chicken + broth	15	6.43	0.2	4.42	5.23	0.0447	30.36	0.086	3.53	7.74	9.65
Canned meat, fish											
Dogfood, beef	28	6.85	1.5	3.96	5.13	0.2255	7.03	0.427	0.70	2.60	9.09
Dogfood, beef	19	6.85	1.5	4.11	5.13	0.0646	22.67	0.122	2.47	7.19	9.24
Dogfood, beef	12	6.85	1.5	4.14	5.32	0.0160	124.64	0.031	9.61	62.20	9.46
Salmon A	28	6.44	1.2	4.11	5.32	0.1826	9.78	0.357	0.84	4.30	9.42
Salmon B	12	6.80	1.1	4.40	4.70	0.0187	331.00	0.033	9.34	277.34	9.10
Lunchmeat A	28	6.50	2.7	3.72	5.20	0.1136	28.88	0.217	1.39	20.08	8.92
Lunchmeat B	19	6.69	2.5	4.05	NG	0.0000					

Values represent averages of 3 replicates of each food.
NG = no growth.

Table 3
Gompertz equation parameters and growth kinetics values for *Shigella flexneri* cultured in milk

	Temperature (°C)	A	C	B	M	EGR [(log CFU/ml)/h]	GT (h)	LPD (h)	MPD (log CFU/ml)
Aerobic growth	28	2.92	6.13	0.1704	8.42	0.384	0.78	2.54	9.05
	19	2.86	6.18	0.0314	43.02	0.071	4.23	11.06	9.05
	15	2.90	5.65	0.0247	57.48	0.051	5.93	16.54	8.55
	12	2.98	5.85	0.0153	230.64	0.033	9.17	165.13	8.83
Anaerobic growth	28	2.74	5.97	0.1765	11.02	0.387	0.78	5.35	8.71
	19	2.94	4.82	0.0734	28.71	0.130	2.32	15.03	7.76
	15	2.73	4.21	0.0250	68.36	0.039	7.77	28.41	6.94
	12	3.02	4.56	0.0104	379.15	0.017	19.40	270.31	7.58
'Stationary' growth	28	3.02	5.62	0.1574	9.13	0.325	0.93	2.77	8.64
	19	3.01	5.02	0.0305	39.31	0.056	5.43	5.96	8.02
	15	2.97	5.05	0.0349	39.41	0.065	4.67	10.59	8.02
	12	3.17	3.97	0.0157	272.88	0.023	13.43	207.48	7.14

Table 4
Comparison of growth of *S. flexneri* observed in milk^a with growth predicted by models

Temperature (°C)	T ₁₀₀₀ (h)				
	Observed			Predicted	
	Aerobic	Anaerobic	Stationary	Aerobic	Anaerobic
28	10.4	13.1	12.1	9.3	15.3
19	53.4	38.3	61.1	68.9	82.7
15	76.0	111.6	58.1	260.9	357.9
12	257.0	462.9	353.9	846.7	> 1000

^aWhole milk, UHT, Grade A, Vit. D, pH 6.50, ~0.5% NaCl.

equation parameters and the derived growth kinetics values for growth in UHT milk are shown in Table 3. The organism grew readily at 15, 19 and 28°C under all conditions of incubation. Growth also occurred at 12°C, with considerably longer lag times in 3 of 3 aerobic, 4 of 9 anaerobic and 3 of 6 'stationary' milk cultures.

Since it is probable that the conditions for bacterial growth in foods under 'stationary' conditions, as described in this work, are not strictly aerobic, the observed growth kinetics values were compared to values predicted by both the aerobic and the anaerobic growth models. The model equations for aerobic growth (Zaika et al., 1992) and anaerobic growth (Zaika et al., 1994) of *S. flexneri* are described in terms of the B and M Gompertz parameters, using four variables: temperature, initial pH, NaCl and NaNO₂ concentrations. These models, incorporated into our Pathogen Modeling Program Version 4.0, were used to calculate the predicted growth kinetics values based on the same temperature, pH, NaCl and NaNO₂ as in the foods tested. For comparison of observed and predicted growth, it is useful to calculate the time required for the bacterial population to increase from 1 to 1000 CFU/g (3 log increase). This value (T₁₀₀₀) can be derived from the Gompertz equation (Table 1).

Table 4 shows a comparison of T₁₀₀₀ values observed for aerobic, anaerobic and 'stationary' growth of *S. flexneri* in milk at 28, 19, 15 and 12°C with values calculated using the growth models. Agreement between observed and predicted T₁₀₀₀ values was good for growth at 28 and 19°C, particularly under aerobic conditions. Very slow growth was predicted by both models at 15 and 12°C. Although observed growth at 12°C was much faster than predicted, it should be noted that some of the replicate cultures failed to grow, and the bacterial population in 5 of 9 replicates under anaerobic conditions and 3 of 6 replicates under 'stationary' conditions decreased to undetectable levels in 260–330 h.

Comparison of observed and predicted T₁₀₀₀ values for growth of *S. flexneri* in canned meat broths is given in Table 5. Agreement between observed values and those predicted by either model was good for growth at 37°C for all three beef broths and at 19°C for broths B and C. Both models predicted no growth at 12°C (T₁₀₀₀ ≥ 1000 h) and no growth was observed in beef broth B. However, the

Table 5

Comparison of growth of *S. flexneri* observed in canned beef and chicken broths with growth predicted by models

	Temperature (°C)	T ₁₀₀₀ (h)		
		Observed	Predicted-aerobic	Predicted-anaerobic
Beef broth A	37	5.4	6.2	7.9
Beef broth B	37	8.0	5.7	8.1
Beef broth C	37	7.8	5.6	6.9
Beef broth A	19	29.5	105.6	85.4
Beef broth B	19	62.4	92.1	79.7
Beef broth C	19	80.7	93.8	88.8
Beef broth A	12	92.4	> 1000	> 1000
Beef broth B	12	N.G.	> 1000	> 1000
Chicken broth	19	38.8	66.8	82.6

Beef broth A: pH 5.90, 0.92% NaCl.

Beef broth B: pH 6.05, 0.69% NaCl.

Beef broth C: pH 6.10, 1.38% NaCl.

Chicken broth: pH 6.60, 0.68% NaCl.

N.G. = no growth.

bacteria grew readily at 12°C in beef broth A, which consistently supported faster growth compared to broths B and C. All three products contained a number of different additives, as listed on the labels, and these undoubtedly have an effect on growth, although they were not taken into account by the growth models. Agreement of observed and predicted growth for canned chicken broth at 19°C was reasonably good, with observed growth being somewhat faster than predicted.

The observed T₁₀₀₀ values for growth of *S. flexneri* in meat and fish products (Table 6) at 37 and 28°C compared favorably with the corresponding values

Table 6

Comparison of growth of *S. flexneri* observed in meat and fish with growth predicted by models

	pH	NaCl (%)	Temperature (°C)	T ₁₀₀₀ (h)		
				Observed	Predicted aerobic	Predicted anaerobic
Chicken and broth	6.43	0.20	37	6.2	5.0	9.6
			15	43.5	269.1	343.9
Canned dogfood	6.85	1.50	28	9.8	9.5	13.5
			19	32.3	71.3	83.6
			12	159.5	881.9	> 1000
Canned salmon, A	6.44	1.22	28	12.8	9.8	13.5
Canned salmon, B	6.80	1.10	12	373.8	813.6	> 1000
Canned lunchmeat A	6.50	2.68	28	34.1	13.3	15.7
Canned lunchmeat B	6.69	2.51	19	N.G.	98.0	111.8

N.G. = no growth.

Table 7
Comparison of growth of *S. flexneri* observed in vegetable products with growth predicted by models

	pH	NaCl (%)	Temperature (°C)	T ₁₀₀₀ (h)		
				Observed	Predicted aerobic	Predicted anaerobic
Vegetable broth	5.48	1.13	37	12.7	8.8	11.1
			19	231.9	167.8	128.1
			12	N.G.	>1000	>1000
Carrots	5.05	0.22	28	N.G.	38.0	58.3
			19	N.G.	334.9	168.1
Peas	6.51	0.00	15	54.4	258.0	357.5
Squash	5.37	0.00	28	14.8	24.8	31.2
			15	231.1	828.3	302.5

N.G. = No growth.

obtained with the aerobic and anaerobic growth models. The bacteria grew faster than predicted in canned dogfood at 19°C; nevertheless, a reasonable estimate for growth was obtained by means of the growth models. Although the predicted T₁₀₀₀ values of 98.0 and 111.8 h for aerobic and anaerobic growth, respectively, suggest that growth should occur in lunchmeat B at 19°C, growth was not observed and the bacterial population decreased ~ 1 log in numbers in 270 h. Failure of the bacteria to grow in this product may have been due to its relatively low water activity ($a_w = 0.961$). Agreement was poor between observed and predicted growth at 12 and 15°C. Considerably faster growth at these temperatures was observed in chicken with broth baby food, canned dogfood and canned salmon B than was predicted by the models.

Comparison of T₁₀₀₀ values for *S. flexneri* cultured in vegetable products with values predicted by the growth models is shown in Table 7. At 37°C, growth observed in canned vegetable broth agreed well with growth predicted with the aerobic and the anaerobic models, while at 19°C observed growth was somewhat slower than predicted. In agreement with predictions obtained with both models, growth did not occur at 12°C and the bacterial population decreased gradually. In some cases only approximate predicted values could be obtained, since the properties of the food were outside the valid range of the models — in the absence or at very low levels of NaCl or at pH < 5.5. Model development was based on data obtained from cultures with a minimum NaCl concentration of 0.5% and a minimum initial pH of 5.5. Even under these limitations, a reasonable estimate of growth can be obtained with the models for growth at the higher temperatures (e.g. squash, 28°C). The T₁₀₀₀ values calculated with the aid of both models suggest that *S. flexneri* should be capable of growing in carrots at 28 and 19°C. However, as shown in Table 2, growth was not observed under these conditions. Instead, the bacterial population decreased to undetectable levels. As mentioned above, antimicrobial substances apparently exist in carrot tissue and are sufficiently active to prevent the growth of *S. flexneri*.

We believe that the data presented indicate that even general growth models, based on a few variables, such as the models for aerobic and anaerobic growth of *S. flexneri* that we developed, can be useful for determining the potential for bacterial growth in foods. Our evaluation of the *S. flexneri* growth models, suggests that the observed and the calculated growth kinetics values compared favorably for growth in the range of 19–37°C, but not for lower temperatures (15 and 12°C), where much slower growth was generally predicted. Factors other than those used in model development, such as food additives or food constituents, may exert an important influence on the growth of the bacterium in specific foods. Poor agreement between the observed and the predicted growth kinetics values at low temperatures is probably due to insufficient growth data at temperatures below 19°C used in model development (Zaika et al., 1992, 1994). Presently we are attempting to remedy this situation by obtaining additional data to improve the accuracy of the models at the lower temperatures.

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