

Effects and Interactions of Temperature, pH, Atmosphere, Sodium Chloride, and Sodium Nitrite on the Growth of *Listeria monocytogenes*

ABSTRACT

The effects and interactions of temperature (5,19,28,37°C), initial pH (6.0 and 7.5), atmosphere (aerobic and anaerobic), sodium chloride content (0.5 and 4.5%), and sodium nitrite concentration (0,50,100,200, 1000 µg/ml) on the growth of *Listeria monocytogenes* Scott A were determined using Tryptose Phosphate Broth. Growth data were analyzed by regression analysis to generate "best-fit" Gompertz equations, which were used subsequently to calculate lag phase duration, exponential growth rate, generation time, and maximum population density values. The data indicated that the growth kinetics of *L. monocytogenes* was dependent on the interaction of the five variables, particularly in regard to exponential growth rates and lag phase durations. The data suggest that sodium nitrite can have significant bacteriostatic activity against *L. monocytogenes* and may provide cured meats with a degree of protection against this microorganism, particularly if employed in conjunction with a combination of acidic pH, vacuum packaging, high salt concentrations, and adequate refrigeration.

The recent realization that foodborne transmission of *Listeria monocytogenes* can play a major role in the etiology of epidemic human listeriosis has led to the need for information on the characteristics of this psychrotrophic pathogen in foods. This includes data on its incidence, growth and survival in meat, poultry, and seafood products. While these products have not been implicated in epidemic foodborne outbreaks of human listeriosis they remain product classes of concern for several reasons including substantial incidence rates of *Listeria* contamination in retail-level samples (3,7,13,14,18,19) and ability of the microorganism to survive or grow under conditions associated with normal processing, distribution, or marketing (6,7,9,15,16,17,20). However, it is logistically impossible to assess the growth of *L. monocytogenes* in the thousands of different product formulations in the marketplace. Gibson et al. (11,12) and Baird-Parker and Kilsby (1) concluded that the only realistic approach for assessing the likely behavior of a pathogen in foods is to identify and characterize the key determinants of the microorganism's growth

and survival in appropriate model systems and prototype foods.

The growth of bacteria in meats and meat products is controlled by the interaction of a number of environmental and nutritional parameters including temperature, pH, oxygen content, sodium chloride concentration, sodium nitrite concentration, etc. (4). Shahamat et al. (20) conducted an assessment of the efficacy of sodium nitrite for the control of *L. monocytogenes* and determined that its activity was dependent on interactions with temperature, pH, and sodium chloride content. They concluded that at the levels permitted in meat products, sodium nitrite would have significant inhibitory activity only in refrigerated products with at least 3% NaCl and a pH of 5.5 or less. However, Shahamat et al. (20) determined the inhibitory activity of nitrite against *L. monocytogenes* by measuring its effect on maximum population densities attained by the microorganism. This approach is useful for measuring bactericidal activity but is less effective for quantifying bacteriostatic activity of the type associated with sodium nitrite. Further, there has been no evaluation of the effects and interactions of oxygen content, a parameter that can impact significantly the inhibitory activity of nitrite against facultative anaerobes such as *Staphylococcus aureus* (5,21). Accordingly, the objective of the current study was to assess quantitatively the effects and interactions of sodium nitrite, pH, temperature, atmosphere, and sodium chloride on the growth kinetics of *L. monocytogenes* in a model system (microbiological medium).

MATERIALS AND METHODS

Microorganism

Listeria monocytogenes Scott A was used throughout the study. Stock cultures were maintained in Brain Heart Infusion Broth (Difco), stored at 4°C and transferred monthly.

Media

Tryptose Phosphate Broth (TPB) (Difco) was used for all experimental cultures. Tryptose Phosphate Agar (TPA) (TPB + 2% agar) was used for all plate counts.

Experimental design

A complete factorial design (5 X 4 X 2 X 2 X 2 X 3) was employed to assess the effects of sodium nitrite concentration (0,50,100,200, and 1000 µg/ml), temperature (5,19,28,37°C), initial pH (6.0 and 7.5), atmosphere (aerobic and anaerobic), and sodium chloride concentration (5 and 45 g/l). All variable combinations were replicated three times, with duplicate samples being taken at each sampling time.

Culturing techniques

TPB was prepared, supplemented with 40 g/l NaCl if appropriate (TPB has a basal NaCl level of 5 g/l), and adjusted to pH 6.0 or 7.5 using 1.0 N HCl or KOH. The medium was then dispensed in 50 ml portions into 250 ml Erlenmeyer flasks (aerobic cultures) or 250 ml trypsinizing flasks with side arm sampling port (anaerobic cultures) (Bellco). The Erlenmeyer flasks were capped with foam plugs, while the trypsinizing flasks were closed with screw-caps and the side arm ports sealed with rubber septa. All flasks were sterilized by autoclaving for 15 min at 15 psi. After cooling, sets of triplicate flasks received 2.0 ml of a filter-sterilized stock solution of sodium nitrite to achieve the desired final concentrations.

All flasks were inoculated with 0.5 ml of a diluted 24 h culture of *L. monocytogenes* to achieve an initial level of approximately 1×10^3 cfu/ml. Prior to inoculation the anaerobic cultures were flushed with sterile N₂ for 10 min. The flasks were then incubated at the appropriate temperature on a rotary shaker (model G-26 Psychrotherm Incubator, New Brunswick Scientific, Inc.) at 150 rpm. Samples were removed periodically from the aerobic and anaerobic cultures by pipette or hypodermic needle and syringe, respectively. The samples were surface plated onto TPA plates using a Spiral Plater (Spiral Systems, Inc.). All plates were incubated for 24 h at 37°C. The pH of the cultures was monitored using the remainder of the plating sample.

Curve fitting and statistical analyses

Growth curves were generated from the experimental data using the Gompertz equation (11,12) (Table 1) in conjunction with ABACUS, a nonlinear regression program that employs a Gauss-Newton integration procedure. (This FORTRAN-based program was developed at this location by W. Damert, and copies

are available upon request.) The four Gompertz parameters were subsequently used to calculate lag phase durations (LPD) (h), exponential growth rates (EGR) (\log_{10} (cfu/ml)/h), generation times (GT) (h), and maximum population densities (MPD) (\log_{10} (cfu/ml)) as described by Gibson et al. (11,12) (Table 1). Cultures displaying less than a 1 log cycle increase over the initial inoculum level were designated as nongrowing and were not submitted to curve fitting. The number of viable cells declined in some cultures after attainment of maximal population densities. This was associated with the fermentative activity of the species (8). In those instances, the population values after initiation of the decline were not considered during curve fitting.

RESULTS

Growth curve data were used to derive "best fit" Gompertz values for each of the variable combinations for aerobic (Table 2) and anaerobic (Table 3) incubation. Each value represents the consolidation of three independent determinations. It was found that growth curves could be fitted well (i.e., have a minimal root mean square error value) with as few as six appropriately dispersed data points (2 each from lag, exponential, and stationary growth phases), though almost all of the Gompertz values were derived from growth curves having 8-12 sampling times. EGR's, GT's, LPD's, and MPD's were calculated subsequently from the Gompertz values.

The Gompertz equation was used also to estimate the combined impact of changes to LPD's and EGR's. This was accomplished by using the values for B, M, and MPD from Tables 2 and 3, and substituting arbitrary set values for L(t). The equation was rearranged and solved for t. For example, Table 4 depicts the estimated time for cultures of *L. monocytogenes* to increase from 1 cfu/ml to 10,000 cfu/ml. This assumed that there was no change in B, M, and MPD as a result of the decreased initial level (A = 0.0), and that C = MPD - A. The validity of this assumption is currently being investigated, but in the meantime these calculations provided a quantitative estimate of the combined bacteriostatic effects of sodium nitrite, indicating that it was most active in the current study when used in combination with anaerobiosis, pH 6.0, 4.5% NaCl, and 5°C incubation.

The five variables interacted to influence the growth of *L. monocytogenes*. This was evidenced largely in changes in LPD and/or EGR. Some suppression of MPD's was observed with non-optimal conditions, particularly in response to the combined effects of sodium nitrite and 5°C incubation (e.g., aerobic/pH 6.0/0.5% NaCl/5°C). However, in most instances if the cultures grew, they ultimately attained MPD's between 10^9 - 10^{11} cfu/ml.

In the absence of sodium nitrite, EGR's (Fig. 1) were affected most strongly by incubation temperature, being maximal at either 37 or 28°C depending on the specific combination of other variables. LPD's were minimal at 37 and 28°C for aerobic and anaerobic cultures, respectively. Sodium chloride had substantially less effect on the microorganisms's growth kinetics. Increased sodium chloride tended to depress EGR's and increase LPD's in the ab-

TABLE 1. Gompertz equation.

$L(t) = A + Ce^{(-B(t-M))}$	
WHERE:	
L(t)	= log count of the number of bacteria at time t (in hours)
A	= asymptotic log count as t decreases indefinitely
C	= asymptotic amount of growth (log number) that occurs as t increases indefinitely
M	= time (in hours) at which the absolute growth rate is maximum
B	= relative growth rate at M
<u>Associated Equations:</u>	
Exponential growth rate (EGR) (\log_{10} cfu/ml/h)	= BC/e
Lag phase duration (LPD) (h)	= M-(1/B)
Generation time (h)	= $\log_{10}(2)e/BC$
Maximum Population Density (MPD) (\log_{10} (cfu/ml))	= A + C

TABLE 2. Gompertz equation parameters and calculated growth curve values for aerobic cultures of *Listeria monocytogenes* Scott A with various combinations of temperature, pH, sodium chloride, and sodium nitrite.

Temp. (°C)	Initial pH	NaCl (%)	NaNO ₂ (µg/ml)	Gompertz Parameters				Growth Rate (Log #/h)	Generation Time (h)	Lag Time (h)	Max. Pop. Density (Log cfu/ml)
				A	C	B	M				
37	6.0	0.5	0	3.72	6.06	0.236	5.41	0.57	0.6	1.2	9.8
			50	3.68	5.89	0.199	5.85	0.43	0.7	0.8	9.6
			100	3.55	5.79	0.201	6.75	0.43	0.7	1.8	9.3
			200	3.55	5.88	0.201	9.24	0.44	0.7	4.3	9.4
			1000	----	NG ^a	0.000	----	0.00	---	---	---
		4.5	3.78	5.68	0.378	9.98	0.79	0.4	7.3	9.5	
		50	3.57	5.58	0.391	9.31	0.80	0.4	6.7	9.1	
		100	3.89	5.23	0.322	10.42	0.62	0.5	7.3	9.1	
		200	3.90	6.45	0.135	19.29	0.32	0.9	11.9	10.3	
		1000	----	NG	0.000	----	0.00	---	----	----	
	7.5	3.65	6.33	0.221	5.22	0.51	0.6	0.7	10.0		
	50	3.64	6.35	0.222	5.19	0.52	0.6	0.7	10.0		
	100	3.46	6.53	0.218	5.44	0.52	0.6	0.8	10.0		
	200	3.30	6.68	0.211	5.44	0.52	0.6	0.7	10.0		
	1000	3.47	6.38	0.237	5.71	0.56	0.5	1.5	9.8		
	4.5	3.56	7.06	0.164	9.42	0.43	0.7	3.3	10.6		
	50	3.75	7.21	0.155	10.48	0.41	0.7	4.0	10.3		
	100	3.73	7.09	0.166	10.32	0.43	0.7	4.3	10.8		
	200	3.64	7.27	0.162	10.73	0.43	0.7	4.5	10.9		
	1000	3.70	7.01	0.164	10.90	0.42	0.7	4.8	10.7		
28	6.0	0.5	0	3.89	5.85	0.265	8.23	0.57	0.5	4.4	9.7
			50	3.80	5.52	0.305	7.89	0.62	0.5	4.6	9.3
			100	3.85	5.58	0.377	8.20	0.77	0.4	5.5	9.4
			200	3.61	6.65	0.081	18.65	0.20	1.5	6.2	10.3
			1000	----	NG	0.000	----	0.00	---	---	----
		4.5	3.81	7.19	0.179	11.74	0.48	0.6	6.2	11.0	
		50	3.88	6.36	0.210	11.76	0.49	0.6	7.0	10.2	
		100	3.83	5.36	0.251	11.29	0.50	0.6	7.3	9.2	
		200	3.86	4.55	0.137	14.84	0.23	1.3	7.5	8.4	
		1000	---	NG	0.000	----	0.00	---	---	---	
	7.5	3.84	6.19	0.210	8.94	0.48	0.6	4.2	10.0		
	50	3.75	6.23	0.209	8.39	0.48	0.6	3.6	10.0		
	100	3.69	6.26	0.184	8.66	0.42	0.7	3.2	9.9		
	200	3.71	6.19	0.197	8.40	0.45	0.7	3.3	9.9		
	1000	3.67	5.94	0.207	7.85	0.45	0.7	3.0	9.6		
	4.5	3.54	7.32	0.126	12.54	0.34	0.9	4.6	10.9		
	50	3.59	7.11	0.129	12.59	0.34	0.9	4.9	10.7		
	100	3.60	7.58	0.117	13.91	0.33	0.9	5.4	11.2		
	200	3.45	6.92	0.116	12.48	0.29	1.0	3.8	10.4		
	1000	3.39	7.08	0.096	13.62	0.25	1.2	3.2	10.5		
19	6.0	0.5	0	3.74	5.62	0.156	20.69	0.32	0.9	14.3	9.4
			50	3.72	5.43	0.225	22.15	0.45	0.7	17.7	9.1
			100	3.67	5.13	0.129	22.31	0.24	1.2	14.6	8.8
			200	3.66	4.70	0.056	26.15	0.10	3.1	8.2	8.3
			1000	---	NG	0.000	----	0.00	---	---	---
	4.5	3.76	5.77	0.056	27.48	0.12	2.5	9.6	9.5		
	50	3.76	4.96	0.056	46.00	0.10	2.9	28.2	8.7		
	100	3.93	4.29	0.069	63.42	0.11	2.8	48.8	8.2		
	200	----	NG	0.000	----	0.00	---	---	---		
	1000	----	NG	0.000	----	0.00	---	---	---		

5	7.5	0.5	0	4.02	6.81	0.100	19.68	0.25	1.2	9.6	10.8	
			50	4.10	6.61	0.100	20.46	0.24	1.2	10.5	10.7	
			100	4.06	6.59	0.122	21.15	0.30	1.0	12.9	10.7	
			200	4.04	6.80	0.096	21.21	0.24	1.3	10.8	10.8	
			1000	3.97	5.86	0.172	22.69	0.37	0.8	16.9	9.8	
		4.5	0	4.13	5.84	0.063	25.98	0.14	2.2	10.0	10.0	
			50	4.10	5.79	0.065	27.10	0.14	2.2	11.7	9.9	
			100	4.08	5.69	0.078	26.66	0.16	1.9	13.7	9.8	
	6.0	0.5	0	3.82	5.00	0.011	131.48	0.02	14.4	43.8	8.8	
			50	3.78	4.52	0.008	174.23	0.01	22.4	50.8	8.3	
			100	3.96	2.91	0.015	246.90	0.02	18.5	181.1	6.9	
			200	----	NG	0.000	-----	0.00	---	----	---	
			1000	----	NG	0.000	-----	0.00	---	----	---	
		4.5	0	3.88	4.66	0.008	211.80	0.01	22.2	85.2	8.5	
			50	3.99	4.78	0.008	300.29	0.01	22.0	172.1	8.8	
			100	3.79	4.62	0.005	417.80	0.01	36.2	213.7	8.4	
			200	----	NG	0.000	-----	0.00	---	----	---	
			1000	----	NG	0.000	-----	0.00	---	----	---	
		7.5	0.5	0	3.97	6.02	0.010	158.50	0.02	13.3	60.5	10.0
				50	3.90	5.94	0.010	158.75	0.02	14.5	53.5	9.8
100	3.87			5.82	0.010	154.65	0.02	14.6	50.5	9.7		
200	3.95			5.54	0.010	152.76	0.02	14.9	51.7	9.5		
1000	3.86			4.41	0.011	141.65	0.02	17.5	47.3	8.3		
4.5	0		3.53	5.51	0.010	175.31	0.02	14.4	78.2	9.0		
	50		3.60	4.77	0.012	171.17	0.02	14.9	84.2	8.4		
	100		3.62	4.64	0.012	165.62	0.02	14.7	82.3	8.3		
	200		3.48	4.78	0.011	158.90	0.02	15.9	66.3	8.3		
	1000		3.67	3.76	0.027	226.21	0.04	7.9	189.7	7.4		

^aNo growth.

TABLE 3. Gompertz equation parameters and calculated growth curve values for anaerobic cultures of *Listeria monocytogenes* Scott A with various combinations of temperature, pH, sodium chloride, and sodium nitrite.

Temp. (°C)	Initial pH	NaCl (%)	NaNO ² (µg/ml)	Gompertz Parameters				Growth Rate (Log #/h)	Generation Time (h)	Lag Time (h)	Max. Pop. Density (Log cfu/ml)
				A	C	B	M				
37	6.0	0.5	0	3.92	5.29	0.307	7.66	0.60	0.5	4.4	9.2
			50	3.77	5.47	0.168	11.31	0.34	0.9	5.3	9.2
			100	3.63	5.96	0.102	14.34	0.22	1.3	4.6	9.6
			200	4.05	4.05	0.216	22.29	0.32	0.9	17.7	8.1
			1000	----	NG ^a	0.000	-----	0.00	---	----	---
	4.5	0	3.65	5.55	0.227	9.48	0.46	0.7	5.1	9.2	
		50	3.80	4.65	0.087	26.44	0.15	2.0	15.0	8.5	
		100	3.71	4.63	0.089	26.46	0.15	2.0	15.2	8.3	
		200	3.79	4.95	0.072	38.28	0.13	2.3	24.4	8.7	
		1000	----	NG	0.000	-----	0.00	---	----	---	
	7.5	0.5	0	3.73	6.04	0.245	6.32	0.54	0.6	2.3	9.8
			50	3.79	5.93	0.256	6.20	0.56	0.5	2.3	9.7
			100	3.82	5.83	0.268	6.18	0.58	0.5	2.4	9.6
			200	3.68	5.99	0.231	6.23	0.51	0.6	1.9	9.7
			1000	4.02	5.45	0.263	8.92	0.53	0.6	5.1	9.5
4.5		0	3.68	5.86	0.327	7.69	0.70	0.4	4.6	9.5	
		50	3.68	5.83	0.239	8.13	0.51	0.6	3.9	9.5	

			100	3.65	5.84	0.332	8.15	0.71	0.4	5.1	9.5
			200	3.72	5.67	0.383	7.12	0.80	0.4	4.5	9.4
			1000	3.78	5.35	0.371	7663	0.73	0.4	5.0	9.1
28	6.0	0.5	0	2.96	6.19	0.195	7.98	0.45	0.7	2.9	9.2
			50	2.76	7.02	0.062	18.68	0.16	1.9	2.7	9.8
			100	3.00	6.35	0.071	21.93	0.17	1.8	7.9	9.3
			200	3.00	6.16	0.069	23.30	0.16	1.9	8.8	9.2
			1000	----	NG	0.000	----	0.00	---	---	---
		4.5	0	3.77	4.96	0.188	11.84	0.34	0.9	6.5	8.7
			50	3.88	4.66	0.161	25.96	0.28	1.1	19.8	8.5
			100	3.97	5.43	0.087	25.95	0.17	1.7	14.5	9.4
			200	3.83	4.45	0.087	44.69	0.14	2.1	33.2	8.3
			1000	----	NG	0.000	----	0.00	---	----	---
	7.5	0.5	0	2.91	6.82	0.198	6.56	0.50	0.6	1.5	9.7
			50	2.96	6.76	0.188	6.84	0.47	0.6	1.5	9.7
			100	3.14	6.65	0.171	7.39	0.42	0.7	1.5	9.8
			200	3.03	6.82	0.157	7.81	0.40	0.8	1.5	9.9
			1000	2.80	7.17	0.121	8.72	0.32	0.9	0.4	10.0
		4.5	0	3.72	5.88	0.221	10.09	0.48	0.6	5.6	9.6
			50	3.68	5.99	0.179	10.98	0.40	0.8	5.4	9.7
			100	3.78	5.89	0.206	10.48	0.45	0.7	5.6	9.7
			200	3.81	5.91	0.154	11.67	0.33	0.9	5.2	9.7
			1000	3.79	5.86	0.115	15.26	0.25	1.2	6.6	9.6
19	6.0	0.5	0	3.48	6.16	0.102	14.15	0.23	1.3	4.3	9.6
			50	3.46	5.32	0.079	25.78	0.15	1.9	13.1	8.8
			100	3.46	5.87	0.034	38.71	0.07	4.1	9.6	9.3
			200	----	NG	0.000	----	0.00	---	---	---
			1000	----	NG	0.000	----	0.00	---	---	---
		4.5	0	3.88	5.79	0.087	20.94	0.19	1.6	9.1	9.7
			50	3.84	4.24	0.096	50.40	0.15	2.0	40.0	8.1
			100	3.82	4.74	0.059	39.46	0.10	2.9	22.5	8.6
			200	----	NG	0.000	----	0.00	---	----	---
			1000	----	NG	0.000	----	0.00	---	---	---
	7.5	0.5	0	3.66	6.07	0.110	12.71	0.25	1.2	3.6	9.7
			50	3.56	6.11	0.108	13.23	0.24	1.2	3.9	9.7
			100	3.58	6.17	0.095	13.98	0.22	1.4	3.4	9.8
			200	3.58	6.17	0.102	13.52	0.23	1.3	3.7	9.8
			1000	3.56	6.28	0.117	13.16	0.27	1.1	4.6	9.9
		4.5	0	4.00	5.86	0.108	20.94	0.23	1.3	11.6	9.9
			50	3.97	6.10	0.089	21.75	0.20	1.5	10.5	10.1
			100	3.94	6.03	0.085	23.57	0.19	1.6	11.8	10.0
			200	4.01	5.88	0.100	23.88	0.22	1.4	13.9	9.9
			1000	3.99	5.17	0.143	23.95	0.27	1.1	16.9	9.2
5	6.0	0.5	0	3.66	5.67	0.036	67.41	0.07	4.1	39.2	9.3
			50	----	NG	0.000	----	0.00	---	----	---
			100	----	NG	0.000	----	0.00	---	----	---
			200	----	NG	0.000	----	0.00	---	----	---
			1000	----	NG	0.000	----	0.00	---	----	---
		4.5	0	3.64	4.11	0.023	173.00	0.03	8.8	129.3	7.8
			50	----	NG	0.000	----	0.00	---	----	---
			100	----	NG	0.000	----	0.00	---	----	---
			200	----	NG	0.000	----	0.00	---	----	---
			1000	----	NG	0.000	----	0.00	---	----	---

7.5	0.5	0	3.69	6.20	0.013	131.54	0.03	10.5	52.2	9.9
		50	3.66	6.20	0.011	147.92	0.03	11.7	59.4	9.9
		100	3.69	6.18	0.010	143.87	0.02	13.5	41.8	9.9
		200	3.79	6.43	0.009	176.31	0.02	14.6	61.4	10.2
		1000	----	NG	0.000	-----	0.00	----	----	----
4.5	0.5	0	3.97	5.53	0.013	137.37	0.03	11.7	58.6	9.5
		50	3.94	5.04	0.013	138.28	0.02	12.8	59.5	9.0
		100	3.94	4.75	0.009	166.79	0.02	19.8	51.8	8.7
		200	3.94	3.56	0.009	239.99	0.01	25.5	128.9	7.5
		1000	----	NG	0.000	-----	0.00	----	----	----

^aNo growth.

TABLE 4. Estimated time (h) for *Listeria monocytogenes* Scott A to increase from 1 to 10,000 cfu/ml.

Atmosphere	Initial pH	NaCl (%)	Temp. (°C)	Sodium Nitrite (µg/ml)				
				0	50	100	200	1000
Aerobic	6.0	0.5	37	6	7	8	10	NG ^a
			28	9	8	9	9	NG
			19	22	23	24	32	NG
			5	152	213	287	NG	NG
			4.5	37	10	10	11	20
	28	12	12	12	17	NG		
	19	30	51	68	NG	NG		
	5	247	331	479	NG	NG		
	7.5	0.5	37	6	6	6	6	6
			28	9	9	9	9	9
			19	20	21	21	21	23
			5	167	169	167	168	172
4.5			37	10	10	10	11	11
28	13	13	14	13	14			
19	27	29	28	31	35			
5	195	198	192	189	244			
Anaerobic	6.0	0.5	37	8	12	16	24	NG
			28	9	21	24	26	NG
			19	15	29	44	NG	NG
			5	72	NG	NG	NG	NG
			4.5	37	10	30	30	42
	28	13	28	28	48	NG		
	19	22	54	44	NG	NG		
	5	192	NG	NG	NG	NG		
	7.5	0.5	37	7	7	7	7	10
			28	7	8	8	9	10
			19	14	14	15	15	14
			5	139	157	154	184	NG
4.5			37	8	9	9	8	8
28	11	12	11	12	16			
19	22	23	25	25	25			
5	149	155	196	292	NG			

^aNo growth.

sence of sodium nitrite, particularly at 19°C. The 37°C/pH 7.5 cultures were an exception; increased EGR's were observed in response to increased sodium chloride. However, this was offset by an increase in LPD's. Anaerobiosis appeared to have had little systematic effect on EGR's and LPD's in the absence of sodium nitrite, but did seem to foster growth of *L. monocytogenes* at the lowest incubation temperature.

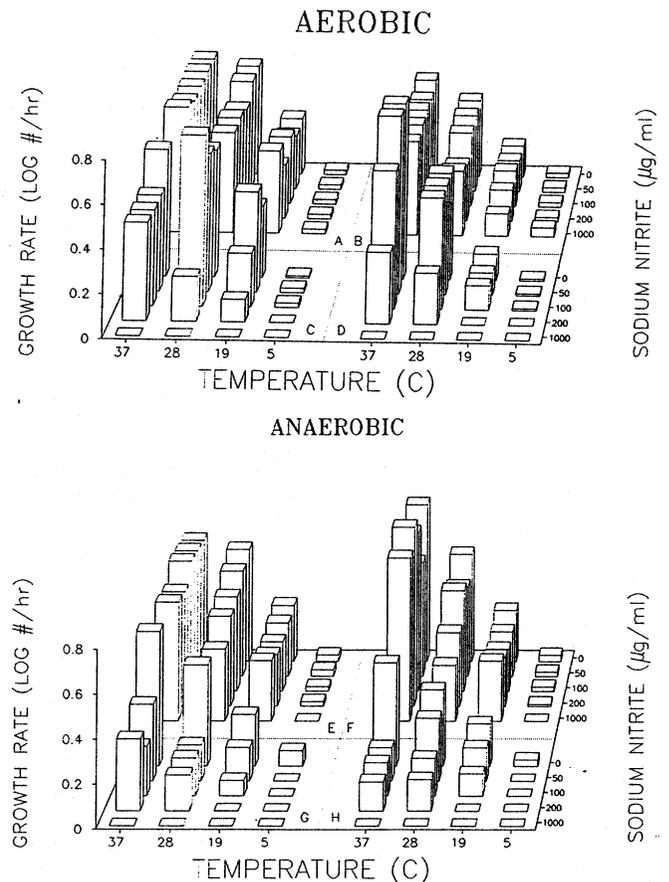


Figure 1. Effects of various sodium nitrite, temperature, pH, and sodium chloride combinations on exponential growth rate ((Log cfu/ml)/h) of *Listeria monocytogenes* cultured aerobically and anaerobically in Tryptose Phosphate Broth.

- A, E = pH 7.5/ 0.5% NaCl
- B, F = pH 7.5/ 4.5% NaCl
- C, G = pH 6.0/ 0.5% NaCl
- D, H = pH 6.0/ 4.5% NaCl

Sodium nitrite displayed both bacteriostatic and bactericidal activity against *L. monocytogenes*. Bacteriostasis typically involved both decreased EGR and extended LPD. Depression of MPD's in response to sodium nitrite treatment was observed; however, this was less important in comparison to the changes to the lag and exponential growth phases. Bactericidal activity (data not shown) was limited largely to a slow die-off of non-growing cultures in association with higher sodium nitrite levels.

The bacteriostatic activity of sodium nitrite was highly dependent on the initial pH of the medium. Essentially no activity was evident in cultures having an initial pH of 7.5, even at 1000 µg/ml which is 6 times the legal limit for sodium nitrite in cured meat products. The only exception was anaerobic/pH 7.5 cultures containing >200 µg/ml. Alternatively, sodium nitrite has substantial bacteriostatic activity when the initial pH of the system was 6.0

Sodium nitrite was more effective against *L. monocytogenes* when the incubation temperature was lowered to 5°C. Anaerobiosis also enhanced bacteriostatic activity of sodium nitrite. Raising the level of sodium chloride from 0.5 to 4.5% also increased the inhibitory effects of sodium nitrite, though the impact was generally less than those observed with anaerobiosis and temperature. The elevated sodium chloride level appeared to have had a greater impact on the activity of sodium nitrite when *L. monocytogenes* was cultured aerobically.

DISCUSSION

The current study demonstrated that sodium nitrite has bacteriostatic activity against *L. monocytogenes* and that this activity is dependent on the temperature, initial pH, sodium chloride concentration, and oxygen content of the environment. Qualitatively, the results were similar to those of Shahamat et al. (20), who reported that sodium nitrite had activity against *L. monocytogenes* and that this was affected by temperature, pH, and sodium chloride content. However, Shahamat et al. (20) only measured the impact of sodium nitrite on MPD; the growth characteristic which the current results indicated was the least affected by sodium nitrite. Accordingly, there are substantial quantitative differences between the studies, with the current study providing growth kinetics data that indicate that legally permitted levels of sodium nitrite (156 µg/g) could have an impact on the growth of *L. monocytogenes* if used in conjunction with specific combinations of the other variables. Further, there were substantial quantitative differences between the two studies in regard to the levels of sodium nitrite needed to prevent growth totally. The current study consistently found that *L. monocytogenes* would not grow at lower sodium nitrite concentrations than those reported by Shahamat et al. (20). These differences likely reflect either variation between the strains used in the studies or the differences in the way sodium nitrite was sterilized. Filter sterilization was selected in the current study based on our assumption that this is a better model for estimating the impact of residual nitrite in meats. While variabil-

ity among strains was not assessed, it is not anticipated that such differences would be large. Overall, the current data indicated that Shahamat et al. (20) significantly underestimated the bacteriostatic activity of sodium nitrite against *L. monocytogenes*.

Oxygen content was found to be an additional factor that must be considered when assessing the growth of *L. monocytogenes* and its sensitivity to the bacteriostatic activity of sodium nitrite. In the absence of sodium nitrite, *L. monocytogenes* displayed characteristics of an organism well adapted to growth in anaerobic environments in that it had roughly equivalent growth kinetics in response to aerobic and anaerobic incubation. In fact at 5°C, anaerobic incubation tended to favor growth of the organism. This suggests that the effectiveness of cold enrichment techniques might be enhanced by conducting it under anaerobic conditions. Anaerobic incubation clearly enhanced the bacteriostatic and bacteriocidal activity of sodium nitrite against the microorganism. This response resembles that observed with *S. aureus* (5,21). If the two species are similar, then it could be anticipated that strict anaerobiosis would enhance further the bacteriostatic activity of sodium nitrite against *L. monocytogenes* as observed with *S. aureus* (10). The current model system study suggests that sodium nitrite is more likely to significantly impact the growth of *L. monocytogenes* in the interior of a cured product or if the product was vacuum packaged. This effect could be expected to be maximal in products that are acidic, have a high level of sodium chloride, and are maintained at the lowest refrigerated storage temperature possible.

In addition to quantifying the bacteriostatic activity of sodium nitrite, the current study also provides quantitative data on the impact of temperature, pH, NaCl, and oxygen content on the growth of *L. monocytogenes*. The data demonstrate clearly that though the microorganism is psychrotrophic, refrigeration is a primary factor controlling the rate of growth. Sodium chloride had a substantially lesser effect on growth, which is in agreement with previous investigators (2,8,20) that have indicated that *L. monocytogenes* has a high degree of tolerance for elevated sodium chloride levels.

The use of the Gompertz equation in conjunction with appropriate curve fitting software proved to be highly effective for generating growth curves and for quantifying the impact of variables on the individual growth phases. The subsequent use of the mathematical expression to effectively integrate of lag phase duration and growth rates to predict expected times for a microorganism to reach target population levels (Table 4) is a powerful tool that should significantly enhance food microbiology. Multidimensional graphs (e.g., Fig. 1) are very helpful in visualizing the relationships among variables.

Quantitative data of this nature can be used to develop efficient models for predicting the behavior of foodborne pathogens; a development that is needed to effectively design food formulations that rely on multiple factors to control the growth of microorganisms. The current data, plus data encompassing additional variable combinations

and multiple inoculum levels, are being used currently to develop a multivariant response surface model for predicting the growth of *L. monocytogenes*. This model will be reported separately.

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