

Response surface models for the growth kinetics of *Escherichia coli* O157:H7

Growth curve data which had been fitted using the Gompertz function were submitted to response surface analysis to develop mathematical models for describing the effects of temperature (10–42°C), initial pH (4.5–8.5), sodium chloride concentration (5–50 g l⁻¹), and oxygen availability (aerobic vs anaerobic) on the growth of a three strain mixture of Escherichia coli O157:H7. Models were developed for the Gompertz B and M terms only after supplemental analyses indicated that the growth kinetics of the organism were independent of inoculum size and that the maximum population density achieved by E. coli were largely independent of the cultural variables. A total of 193 aerobic and 145 anaerobic growth curves representing 84 and 71 distinct variable combinations, respectively, were evaluated, with separate models being generated for the aerobic and anaerobic data. The data were analyzed using natural logarithm and square root transformations in combination with quadratic and cubic models. Subsequent evaluations indicated that the most effective response surface models were those based on a logarithmic transformation in combination with a quadratic model. These models provide a rapid means of estimating the effects of the four variables on the growth of E. coli O157:H7.

Introduction

Escherichia coli O157:H7 is the principal serovar of this species responsible for haemorrhagic colitis and haemolytic uremic syndrome. The etiology of these diseases commonly involves foodborne or waterborne transmission, with ground beef being the food most often implicated (Riley 1987, Belongia et al. 1991, Dev et al. 1991, Doyle 1991, Wells et al. 1991). The severity of the diseases

associated with *E. coli* O157:H7 makes it imperative that its presence, and particularly its growth in food products be controlled. Research with O157:H7 and other serovars of *E. coli* indicate that the organism should be susceptible to commonly employed processing and sanitation protocols, and that food formulation and storage parameters can be manipulated to prevent its growth. However, the latter approach is hampered by a lack of quantitative data on the effects of food-associated variables on the growth kinetics of the serovar.

Recently, we (Buchanan and Klawitter 1992) reported on the effects of incubation temperature (5–42°C), initial pH (4.5–8.5), sodium chloride content

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(5–50 g l⁻¹), and atmosphere (aerobic vs anaerobic) on the growth of a three strain mixture of *E. coli* O157:H7. The purpose of the current manuscript is to describe the utilization of that data to develop response surface models that allow the growth kinetics of the pathogen to be estimated for any combination of the four variables within the specified ranges.

Materials and Methods

Experimental design

A fractional factorial design was used to determine the effects and interactions of incubation temperature (5–42°C), initial pH (4.5–8.5) and sodium chloride content (5–50 g l⁻¹). Separate databases were generated to assess the effect of oxygen availability (aerobic vs anaerobic).

Micro-organisms

A mixture of three *Escherichia coli* O157:H7 strains, 933, 45753-35 and A9218-C1, was used throughout the modeling phases of the study. Inocula were prepared as described by Buchanan and Klawitter (1992). *E. coli* 933 alone was used in the verification studies with foods.

Culture techniques

The mixture of O157:H7 strains was cultured in Brain Heart Infusion broth (Difco) as described by Buchanan and Klawitter (1992). Briefly, the broth was supplemented with the necessary level of sodium chloride, brought up to volume, adjusted to the desired pH, transferred in 50 ml portions to either 250 ml Erlenmeyer (aerobic) or 250 ml trypticizing (anaerobic) flasks, and autoclaved. Each flask was inoculated with 0.5 ml of a diluted mixture of the three O157:H7 strains (target inoculum = 10³ cfu ml⁻¹). The trypticizing flasks were flushed with sterile N₂ and sealed with a rubber stopper and septum. All flasks were incubated at the appropriate temperature on rotary shakers (150 rpm). Samples were removed periodically and enumerated on Brain Heart Infusion agar using a spiral plater. Conditions that did not support growth (i.e. increase of at least 1 log cycle) after ≥250 h were classified as non-growing.

Food samples

Three containers of each of four products, canned tunafish, canned dogfood, UHT milk and canned chicken broth, were purchased from a local supermarket. The contents were aseptically transferred to either sterile 500 ml beakers (tuna and dogfood) or sterile 250 ml Erlenmeyer flasks (milk and chicken broth), with a small portion being reserved for pH and water activity determinations. The pH of milk and chicken broth was determined directly using a pH meter (model 501, Orion). A 5.0 g portion of tuna or dogfood was mixed with 5.0 ml of distilled/deionized water, mixed for 1.0 min with a vortex mixer. The aqueous phase was decanted and measured with a pH meter. Water activity was determined by assaying 5.0 g portions of tuna or dogfood and 5.0 ml portions of milk or chicken broth using a water activity meter (model CX-2, Aqua Lab).

The milk and chicken broth were inoculated with a diluted culture of *E. coli* O157:H7-933 as described above to achieve an inoculum of 10²–10³ cfu ml⁻¹. For the tuna and dogfood, the final dilution of the starter culture was performed using diluent containing green food coloring. The foods were then inoculated to achieve a target level of 10³–10⁴ cfu g⁻¹ and mixed until the green dye was evenly distributed throughout the food sample. The dogfood, UHT milk, chicken broth and tunafish were incubated aerobically without agitation at 12°, 19°, 28° and 42°C, respectively. Samples were removed periodically, diluted in sterile 0.1% peptone water and enumerated on MacConkey agar plates.

Curve fitting

Growth curves were generated by fitting the Gompertz function (Gibson et al. 1988) to the plate count data using ABACUS, a non-linear curve-fitting program developed by W. Damert (USDA ARS Eastern Regional Research Center) that uses a Gauss-Newton iterative procedure. Typically, the Gompertz A value was fixed at the experimental value obtained for the 0 h sample. While this introduces a small systematic error (Garthright 1991), experience has indicated that the impact is negligible while greatly facilitating curve fitting. Once generated, the Gompertz parameters were used to calculate the cultures' lag phase duration (LPD), exponential growth rate (EGR), generation time (GT)

and maximum population density (MPD) as described previously (Buchanan and Klawitter 1992).

Model generation

Transformations of the Gompertz *M* and *B* values were modeled on the independent variables (temperature, pH, NaCl) by response surface analysis using quadratic and cubic polynomial models. These and additional statistical analyses were performed using SAS/STAT (1989).

Results

The databases of Buchanan and Klawitter (1992), along with the additional data in Table 1, were used for model development. This represents a total of 193 aerobic and 145 anaerobic growth curves, encompassing 84 and 71 distinct variable combinations, respectively. Space limitations preclude inclusion of the entire database; however, it is available to interested parties upon request.

Prior to model development, the effects of the independent variables on MPD, and the effect of inoculum size on the serovar's growth kinetics were examined in a series of supplemental evaluations. As reported by Buchanan and

Klawitter (1992), the MPD attained by the organism was largely independent of the cultural variables. If the serovar grew, it typically achieved an MPD that varied randomly between 10^8 and 10^{10} cfu ml⁻¹. Only when two or more variables approached values that would not support growth was there any systematic depression of MPD below 10^8 cfu ml⁻¹.

The effect of inoculum size on the growth kinetics of *E. coli* O157:H7 was evaluated using two aerobic variable combinations: (1) 28°C, pH 7.2, 0.5% NaCl; and (2) 19°C, pH 7.0, 5.0% NaCl. These combinations were selected to reflect near optimal conditions versus an environment where two variables were non-optimal. An inoculum range of between approximately 80 and 800 000 cfu ml⁻¹ was examined (Fig. 1). Regression analysis indicated that there was no significant effect on LPD, GT or MPD related to inoculum size.

Based on the two groups of observations above, models were developed for the Gompertz *B* and *M* terms, but not *A* and *C*. These observations and the corresponding assumptions related to model development are similar to those

Table 1. Additional data appended to that of Buchanan and Bagi (1992) for use as the basis for development of response surface models for *Escherichia coli* O157:H7.

Independent variables				Gompertz parameters							
TEM	pH	NaCl	ATM	A	C	B	M	LPD	EGR	GT	MPD
5	4.5	5	A	2.80	NG	0.0000	—	—	0.000	—	—
5	4.5	50	A	2.61	NG	0.0000	—	—	0.000	—	—
5	5.5	50	A	2.61	NG	0.0000	—	—	0.000	—	—
5	6.5	50	A	2.33	NG	0.0000	—	—	0.000	—	—
5	7.5	50	A	2.33	NG	0.0000	—	—	0.000	—	—
5	8.5	50	A	2.41	NG	0.0000	—	—	0.000	—	—
12	5.5	50	A	3.16	NG	0.0000	—	—	0.000	—	—
12	6.5	5	A	3.03	6.93	0.0280	54.40	18.7	0.071	4.2	9.96
12	8.5	50	A	3.10	NG	0.0000	—	—	0.000	—	—
12	6.5	5	N	3.06	6.42	0.0279	51.57	15.7	0.066	4.2	9.48

Abbreviations: TEM, temperature (°C); NaCl, sodium chloride (g l⁻¹); ATM, atmosphere (A, aerobic; N, anaerobic); LPD, lag phase duration (h); EGR, exponential growth rate [(log (cfu ml⁻¹))h⁻¹]; GT, generation time (h); MPD, maximum population density [log (cfu ml⁻¹); NG, no growth.

reached by others that have employed this approach (Gibson et al. 1988, Buchanan and Phillips 1990, Palumbo et al. 1991, 1992, Zaika et al. 1992).

The B and M values were evaluated using two transformations: (1) $\ln(B)$ and $\ln(M)$; and (2) $(B)^{0.5}$ and $(1/M)^{0.5}$.

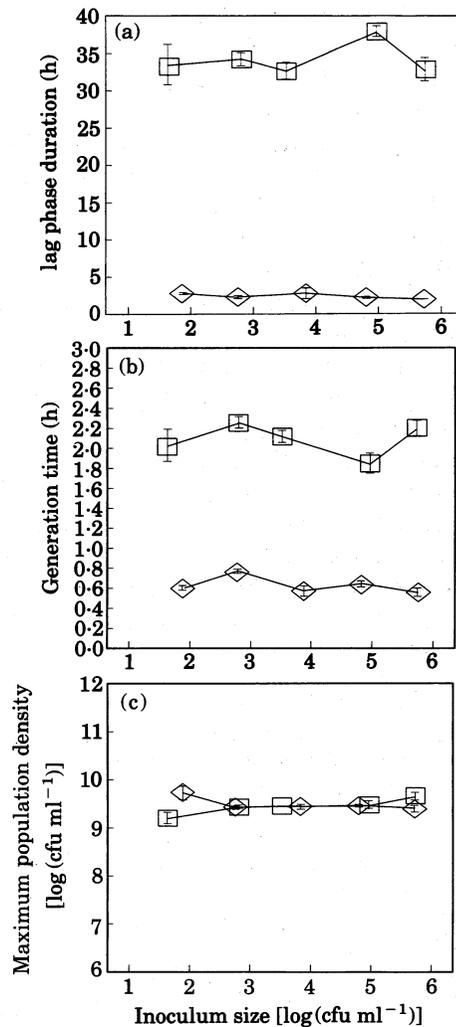


Fig. 1. The effect of inoculum size on the lag phase duration (a), generation time (b) and maximum population density (c) for a three strain mixture of *Escherichia coli* O157:H7 cultured under near optimal (28°C, pH 7.2, 0.5% NaCl; ◇) and somewhat restrictive (19°C, pH 7.0, 5.0% NaCl; □) conditions. Error bars indicate \pm one s.d.

The no-growth data are excluded from the analyses using the logarithmic transformation. The square root transformation was evaluated as an alternate means for stabilizing the variance while permitting inclusion of no-growth data. The reciprocal of M was employed with the assumption that in no growth conditions, $M = \infty$ and $1/M = 0$. Models were developed in four iterations, with additional data being generated after each iteration in the areas of the multi-dimensional surface where the observed vs predicted fit was weak or nonsensical. Both quadratic and cubic models were evaluated on the basis of R^2 values, residuals, and how well the derived values for LPD and GT fit the observed data. The final versions of the quadratic models are presented in Table 2. The cubic models (not shown) offered no clear advantage over the simpler quadratic models and are not discussed further.

Both the natural logarithm and square root transformations provided models that gave good fits based on the adjusted R^2 values (Table 2) and comparison of residuals. Comparison of derived values for GT and LPD with those observed experimentally (Tables 3 and 4) indicated that the two transformations yielded models that gave reasonable values for the derived growth kinetics over much of the range where the organism grew well. However, as might be expected based on the inclusion or exclusion of the no-growth data, the two models had greater divergence as conditions became non-optimal. The models based on natural logarithm transformations tended to underestimate the impact of inhibitory conditions, whereas the square root transformation tended to overestimate these effects. The anaerobic models based on square root transformations were also plagued with anomalous regions that yielded negative LPD values. This is one of the limita-

Table 2. Quadratic response surface models of the Gompertz *B* and *M* values for the growth of *Escherichia coli* O157:H7 as a function of incubation temperature, initial pH and sodium chloride content. [(*T*, °C)^b; *P*, pH (4.5–8.5); and *S*, NaCl (5–50 g l⁻¹).

Aerobic

Logarithmic (natural) transformation

$$\ln(B) = -11.9212 + 0.2407*T + 1.8524*P - 0.0657*S + 0.000938*TP - 0.000125*TS + 0.00386*PS - 0.000295*T^2 - 0.1373*P^2 + 0.000489*S^2$$

Adjusted $R^2 = 0.857^a$

$$\ln(M) = 13.5304 - 0.2544*T - 2.0912*P + 0.0370*S - 0.00449*TP + 0.000528*TS - 0.00329*PS + 0.00342*T^2 + 0.1648*P^2 + 0.0000402*S^2$$

Adjusted $R^2 = 0.922$

Square root transformation

$$B^{0.5} = -1.2299 + 0.0223*T - 0.3506*P - 0.00416*S + 0.00182*TP - 0.000171*TS + 0.000315*PS - 0.000316*T^2 - 0.0287*P^2 + 0.0000550*S^2$$

Adjusted $R^2 = 0.875$

$$(1/M)^{0.5} = -0.9272 + 0.0175*T + 0.2564*P - 0.000138*S + 0.00119*TP - 0.000194*TS + 0.0000697*PS - 0.000209*T^2 - 0.0206*P^2 + 0.0000143*S^2$$

Adjusted $R^2 = 0.915$

Anaerobic

Logarithmic (natural) transformation

$$\ln(B) = -11.7062 + 0.2588*T + 1.7679*P - 0.0374*S + 0.0056*TP + 0.0000547*TS + 0.00571*PS - 0.00362*T^2 - 0.1504*P^2 - 0.000528*S^2$$

Adjusted $R^2 = 0.866$

$$\ln(M) = 15.5302 - 0.2802*T - 2.6383*P + 0.0209*S - 0.00920*TP + 0.0000387*TS + 0.000409*PS + 0.00469*T^2 + 0.2119*P^2 + 0.000212*S^2$$

Adjusted $R^2 = 0.935$

Square root transformation

$$B^{0.5} = -0.8972 + 0.0208*T + 0.2563*P - 0.00176*S + 0.000124*TP - 0.000199*TS + 0.000657*PS - 0.000151*T^2 - 0.0219*P^2 - 0.0000313*S^2$$

Adjusted $R^2 = 0.908$

$$(1/M)^{0.5} = -0.9733 + 0.0156*T + 0.2455*P + 0.00115*S + 0.00132*TP - 0.000165*TS - 0.000135*PS - 0.000211*T^2 - 0.0198*P^2 + 0.00000428*S^2$$

Adjusted $R^2 = 0.936$

^aAdjusted $R^2 = R^2/\text{Max } R^2$ (Draper and Smith 1981).

^b5–42°C for square root-based models; 10–42°C for ln-based models.

tions of response surface analysis; but can often be overcome by the inclusion of additional variable combinations. Overall, the quadratic models using a ln transformation provided a more useful tool for predicting the behavior of *E. coli* O157:H7. The fit between observed and predicted values (no-growth data excluded) is depicted graphically in Fig. 2. The comparison in Tables 3 and 4 were calculated using fixed values for *A*

and *C*. Growth curves corrected for other inoculum levels can be generated using the approach of Buchanan (1991).

Comparison of the F-values associated with the *B* and *M* terms of the aerobic and anaerobic models based on ln transformations (Table 5) indicated that the majority of the organism's response could be attributed to the three primary variables. There was relatively little effect attributable to the cross product

Table 3. Comparison of observed vs predicted lag phase duration (h) and generation time (h) values for aerobic cultures of *Escherichia coli* O157:H7. Predicted values were derived from quadratic models based on ln transformation of *B* and *M* and square root transformations of *B* and *1/M*. Value of 6.34 was assumed for the Gompertz *C* term.

TEM (°C)	pH	NaCl (g l ⁻¹)	Observed		Predicted ln-transformation		Predicted SQRT- transformation	
			GT	LPD	GT	LPD	GT	LPD
5	4.5	5	No growth		30.0	309.6	11.6	48.1
5	4.5	50	No growth		80.9	1203.3	7.5	79.0
5	5.5	5	No growth		18.1	194.2	132.9	209.1
5	5.5	50	No growth		41.1	656.0	70.6	980.8
5	6.5	5	No growth		14.4	175.6	632.3	1099.1
5	6.5	50	No growth		27.5	508.6	942.8	13412.6
5	7.5	5	No growth		15.1	226.0	43.0	337.8
5	7.5	50	No growth		24.2	555.4	89.1	457.4
5	8.5	50	No growth		28.0	846.2	8.7	47.5
8	5.5	5	No growth		9.7	91.4	49.9	551.0
8	6.0	20	No growth		13.4	102.6	103.7	79.6
8	6.5	5	No growth		7.7	81.4	24.1	187.1
8	7.0	20	No growth		11.5	107.1	114.1	25.4
8	7.5	5	No growth		8.1	103.9	88.7	454.2
8	8.5	5	No growth		11.1	186.7	43.7	539.9
10	4.5	5	No growth		11.0	94.4	356.1	10634.4
10	5.5	5	4.6	38.4	6.6	57.2	12.3	101.4
10	5.5	20	No growth		11.0	73.4	34.7	111.8
10	6.5	5	6.2	44.2	5.2	50.4	7.8	55.6
12	5.5	5	5.1	24.7	4.6	36.8	5.6	41.7
12	5.5	20	6.3	48.2	7.7	48.2	11.7	56.4
12	5.5	50	No growth		10.9	164.1	17.5	265.3
12	6.0	5	5.0	21.9	4.0	32.8	4.3	30.4
12	6.0	20	5.5	30.2	6.4	42.3	7.7	40.2
12	6.5	20	5.2	26.9	5.7	40.9	6.7	35.3
12	6.5	35	19.6	173.1	7.2	66.7	8.7	59.5
12	6.5	50	No growth		7.2	123.0	7.7	40.2
12	7.0	20	5.3	27.1	5.5	43.4	7.1	37.0
12	7.5	5	5.0	71.9	3.8	40.4	5.5	33.4
12	7.5	50	No growth		6.3	129.6	9.6	180.3
12	8.0	20	2.8	79.3	6.2	63.6	18.7	75.8
12	8.5	5	5.1	46.6	5.2	72.1	27.9	101.6
12	8.5	50	No growth		7.3	191.4	63.4	18500.0
19	4.5	5	2.5	17.5	2.6	17.5	3.1	19.6
19	4.5	20	11.8	83.5	4.7	26.0	7.2	37.2
19	4.5	35	No growth		6.7	52.7	16.1	127.7
19	4.5	50	No growth		7.7	119.0	26.5	1023.3
19	5.0	20	2.6	10.4	3.4	18.1	3.4	20.1
19	5.5	5	1.2	12.2	1.6	9.8	1.4	9.0
19	5.5	50	3.3	37.5	3.9	59.4	3.8	61.0
19	6.0	20	1.2	17.2	2.2	11.9	1.8	11.2
19	6.5	5	1.2	9.7	1.2	8.7	1.1	6.8
19	6.5	20	1.4	12.9	2.0	11.3	1.6	10.1

Table 3. Continued.

TEM (°C)	pH	NaCl (g l ⁻¹)	Observed		Predicted ln-transformation		Predicted SQRT- transformation	
			GT	LPD	GT	LPD	GT	LPD
19	6.5	35	2.5	16.0	2.5	20.8	2.1	17.6
19	6.5	50	5.3	21.7	2.6	42.4	2.2	33.5
19	7.0	20	1.1	15.8	1.9	11.7	1.6	10.0
19	7.0	50	2.1	34.2	2.3	40.9	2.1	32.8
19	7.2	5	1.2	10.6	1.2	9.0	1.1	6.8
19	7.5	5	1.2	9.2	1.3	10.0	1.2	7.2
19	7.5	50	4.7	105.7	2.2	42.9	2.3	37.9
19	8.0	20	2.6	17.1	2.1	16.8	2.1	13.4
28	4.5	5	0.7	5.0	1.0	6.0	1.1	6.0
28	4.5	20	1.1	7.0	1.8	10.3	2.2	12.1
28	4.5	35	10.5	5.2	2.7	23.6	4.1	38.3
28	4.5	50	2.3	41.9	3.1	57.1	7.2	236.0
28	5.5	5	0.5	1.8	0.6	3.1	0.6	3.6
28	5.5	50	1.1	42.5	1.6	27.0	1.9	32.7
28	6.5	5	0.5	3.0	0.5	2.4	0.5	2.9
28	6.5	20	0.6	4.0	0.8	3.8	0.7	4.6
28	6.5	35	0.7	7.6	1.0	8.1	0.9	8.7
28	6.5	50	1.0	16.1	1.0	18.2	1.1	18.4
28	7.0	35	0.7	4.8	0.9	7.9	0.9	8.3
28	7.2	5	0.6	2.4	0.5	2.5	0.5	2.8
28	7.5	5	0.6	2.8	0.5	2.7	0.5	2.8
28	7.5	50	0.9	18.2	0.9	17.4	1.0	17.8
28	8.0	20	0.6	2.2	0.8	5.1	0.7	5.1
28	8.5	5	0.7	2.9	0.6	4.7	0.6	3.4
28	8.5	35	1.2	7.6	1.1	12.5	1.2	12.4
37	4.5	5	0.7	2.5	0.6	4.0	0.7	3.2
37	4.5	50	No growth		2.1	48.5	6.0	288.1
37	5.5	5	0.4	1.0	0.4	1.9	0.4	2.1
37	5.5	50	1.4	27.7	1.0	21.9	1.5	31.3
37	6.5	5	0.3	1.4	0.3	1.3	0.3	1.7
37	6.5	50	0.8	10.5	0.7	14.0	0.9	16.0
37	7.5	5	0.3	1.9	0.3	1.4	0.3	1.7
37	7.5	50	0.7	7.2	0.6	12.7	0.8	14.0
37	8.5	5	0.3	2.2	0.4	2.2	0.4	1.9
37	8.5	50	0.6	25.5	0.6	16.2	0.9	18.6
42	4.5	5	0.7	5.2	0.6	4.2	0.7	2.5
42	6.5	5	0.3	1.5	0.3	1.3	0.3	1.4
42	6.5	50	0.8	26.3	0.6	15.4	0.9	17.2
42	8.5	5	0.3	1.8	0.4	2.0	0.3	1.5
42	8.5	50	No growth		0.6	16.8	0.8	17.6

terms, suggesting that the three primary variables are largely independent. This is in general agreement with McMeekin et al. (1987) and Chandler and McMeekin (1989) who reported that

the effects of temperature and water activity on *Staphylococcus xylosus* were independent, and Adams et al. (1991) who found that effects of temperature and pH were independent for *Yersinia*

Table 4. Comparison of observed vs predicted lag phase duration and generation time values for anaerobic cultures of *Escherichia coli* O157:H7. Predicted values were derived from quadratic models based on ln transformations of *B* and *M*, and square root transformations of *B* and *1/M*. A value of 5.70 was assumed for the Gompertz *C* term.

TEM (°C)	pH	NaCl (g l ⁻¹)	Observed		Predicted ln-transformation		Predicted SQRT- transformation	
			GT	LPD	GT	LPD	GT	LPD
5	5.50	5	No growth		26.7	225.1	974.6	-2258.7
5	6.50	5	No growth		26.2	175.4	1709.3	770652.2
5	7.50	5	No growth		34.7	233.9	57.9	981.7
8	5.50	5	No growth		12.9	93.4	37.9	281.0
8	6.00	20	No growth		15.5	138.8	31.6	306.5
8	6.50	5	No growth		12.4	68.5	30.9	58.2
8	7.00	20	No growth		15.9	148.6	37.2	379.9
8	7.50	5	No growth		16.2	87.7	146.8	-352.7
8	8.50	5	No growth		28.5	197.3	59.1	1562.7
10	4.50	5	No growth		11.6	124.5	45.2	2353.5
10	5.50	5	9.7	39.5	8.2	54.7	12.1	75.4
10	5.50	20	No growth		10.9	96.7	15.8	148.2
10	6.50	5	6.8	48.7	7.8	38.5	10.3	29.5
12	5.50	5	4.5	25.9	5.4	33.4	5.9	35.7
12	5.50	20	6.1	38.3	7.1	59.9	7.8	63.9
12	6.00	5	4.6	22.8	5.0	25.7	5.2	22.9
12	6.00	20	3.9	43.8	6.4	49.6	6.2	43.4
12	6.50	20	5.1	21.3	6.2	46.9	5.8	37.9
12	6.50	35	17.1	296.3	9.5	87.4	8.1	64.6
12	6.50	50	No growth		18.5	159.6	15.7	89.7
12	7.00	20	4.7	34.0	6.4	50.7	6.2	41.2
12	7.50	5	5.8	35.2	6.5	27.1	7.9	14.4
12	7.50	50	No growth		18.2	238.8	16.6	312.3
12	8.00	20	7.5	58.8	8.7	87.0	12.4	115.4
12	8.50	5	8.2	49.5	11.2	59.8	40.5	-39.7
19	4.50	5	4.1	17.7	2.3	22.6	2.4	27.4
19	4.50	20	9.0	74.9	3.3	36.6	3.8	51.4
19	4.50	35	No growth		6.0	59.2	8.5	105.3
19	4.50	50	No growth		13.8	92.2	53.7	130.2
19	5.00	20	2.4	16.3	2.5	22.7	2.6	24.8
19	5.50	5	1.2	8.8	1.6	8.6	1.5	9.8
19	5.50	50	3.1	41.9	7.2	46.1	6.8	34.7
19	6.00	20	1.7	11.8	1.8	12.5	1.7	12.3
19	6.50	5	1.4	6.4	1.4	5.0	1.3	6.2
19	6.50	20	1.3	14.2	1.7	11.3	1.6	10.8
19	6.50	35	2.5	16.0	2.6	21.6	2.2	17.5
19	6.50	50	6.4	15.2	5.0	39.8	3.8	27.3
19	7.00	20	1.2	17.2	1.7	11.7	1.6	10.6
19	7.50	5	1.3	5.7	1.7	5.3	1.5	5.7
19	7.50	50	12.3	57.5	4.8	55.8	3.6	41.1
19	8.00	20	2.6	20.8	2.3	18.8	2.1	14.8
28	4.50	5	0.9	7.3	0.8	9.8	0.9	10.3
28	4.50	20	0.5	17.2	1.2	16.0	1.3	19.2

Table 4. Continued.

TEM (°C)	pH	NaCl (g l ⁻¹)	Observed		Predicted ln-transformation		Predicted SQRT- transformation	
			GT	LPD	GT	LPD	GT	LPD
28	4.50	35	5.2	12.1	2.1	26.4	2.5	41.4
28	4.50	50	7.4	57.5	4.8	43.4	7.8	123.5
28	5.50	5	0.4	2.8	0.5	3.5	0.6	4.8
28	5.50	50	2.5	22.6	2.4	19.6	2.5	25.6
28	6.50	5	0.6	2.6	0.5	1.9	0.5	3.3
28	6.50	20	0.7	3.2	0.5	4.1	0.7	5.5
28	6.50	35	0.7	6.0	0.8	7.9	1.0	9.3
28	6.50	50	0.8	16.9	1.6	14.9	1.6	16.5
28	7.00	35	0.6	5.6	0.8	8.0	0.9	8.9
28	7.50	5	0.6	2.3	0.5	1.8	0.6	3.0
28	7.50	50	0.7	18.3	1.4	18.4	1.4	18.1
28	8.00	20	0.7	2.9	0.7	5.8	0.8	5.8
28	8.50	5	0.7	2.8	0.8	3.1	0.7	3.4
28	8.50	35	0.6	18.9	1.1	17.3	1.1	14.5
37	4.50	5	0.6	6.6	0.5	9.7	0.5	7.0
37	4.50	50	15.5	46.4	3.1	46.2	3.8	171.1
37	5.50	5	0.4	2.4	0.3	3.5	0.4	3.6
37	5.50	50	1.0	15.7	1.4	19.2	1.5	28.4
37	6.50	5	0.3	1.7	0.3	1.9	0.3	2.5
37	6.50	50	1.3	12.7	0.9	12.9	1.0	15.7
37	7.50	5	0.4	3.0	0.3	1.6	0.3	2.2
37	7.50	50	0.9	11.7	0.8	14.0	0.9	14.6
37	8.50	5	0.4	2.1	0.4	2.5	0.4	2.3
42	4.50	5	0.6	23.8	0.5	13.4	0.4	6.5
42	6.50	5	0.2	1.8	0.3	2.5	0.3	2.3
42	8.50	5	0.3	2.7	0.4	3.1	0.3	2.1

enterocolitica. However, the large F-values associated with the T^2 and P^2 terms suggest a strong quadratic dependency for those variables.

Since no quantitative data on the growth of *E. coli* O157:H7 in foods were available to assess the ability of the models to predict the pathogen's growth kinetics in food systems, the organism was cultured in four foods, UHT milk, canned tunafish, canned chicken broth and canned dogfood (Table 6). Additionally, reported generation times for non-pathogenic *E. coli* strains on raw comminuted mutton (Smith 1985) and beef slices (Shaw and Nicol 1969) were compared. The models were solved using

two estimates for NaCl concentration; one based on assumed level of salt, and the other based on adding sufficient NaCl to achieve the experimentally observed water activities. The models provided very reasonable estimates for both the reported data for non-pathogenic strains and the growth of O157:H7 in the four products evaluated in the current study. The predictions more closely matched the observed food data when the NaCl values used were those levels assumed for the product, as compared to increasing the NaCl level to match the observed water activity. There was good agreement with values for tryptic soy broth (Glass et al. 1992).

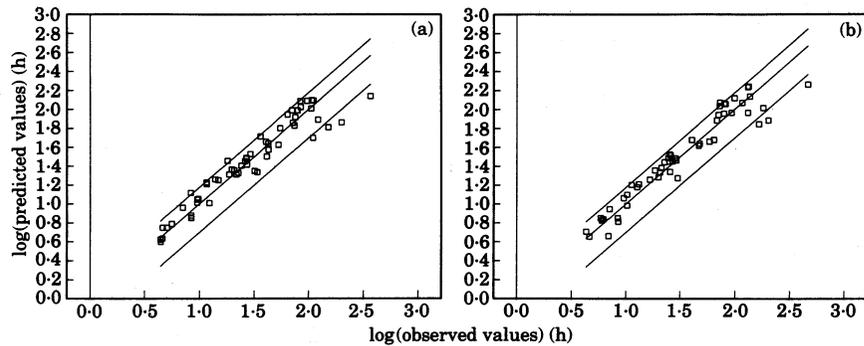


Fig. 2. Estimated times to achieve a 1000-fold increase in population density based on experimental data compared with predictions by the ln-transformation response surface models. 'No growth' data not included. (a) aerobic (b) anaerobic. Centre line is the line of identity and the two exterior lines present $\pm 50\%$ of observed values.

Table 5. F-values for independent variables and their cross products for the quadratic models based on ln transformations.

	Aerobic		Anaerobic	
	<i>B</i>	<i>M</i>	<i>B</i>	<i>M</i>
<i>T</i>	608.6 ^a	943.3 ^a	486.8 ^a	807.1 ^a
<i>P</i>	70.8 ^a	74.0 ^a	9.4 ^b	73.8 ^a
<i>S</i>	73.2 ^a	464.9 ^a	82.7 ^a	304.5 ^a
<i>T*P</i>	1.3	0.3	1.8	12.7 ^b
<i>T*S</i>	2.9	25.5 ^a	0.9	2.4
<i>P*S</i>	1.9	0.3	9.3 ^b	0.3
<i>T²</i>	46.0 ^a	102.0 ^a	48.0 ^a	156.4 ^a
<i>P²</i>	42.3 ^a	79.2 ^a	27.7 ^a	125.7 ^a
<i>S²</i>	7.7 ^b	0.1	6.0	2.1

^a $P \leq 0.0001$.

^b $0.01 > P > 0.0001$.

Discussion

This study expands work by our laboratory directed toward the acquisition of quantitative data on the growth kinetics of selected foodborne pathogens, and the subsequent development of mathematical models that can be used to estimate the bacteria's behaviour in foods (Buchanan et al. 1989, Buchanan and Phillips 1990, Palumbo et al. 1991,

1992, Zaika et al. 1989, 1991, 1992, Buchanan and Klawitter 1992, Benedict et al. 1993). These studies have provided extensive quantitative kinetics data that has challenged a number of the assumed growth characteristics of vegetative pathogenic bacteria. For example, the lack of effect of inoculum size over a broad range noted in the current study with *E. coli* has also been observed with *Listeria monocytogenes*, *Staphylococcus aureus*, *Shigella flexneri* and *Aeromonas hydrophila*. The current study also supports the observation that the MPD for foodborne pathogens is largely independent of cultural and environmental parameters over much of their growth range. However, this latter characteristic does not appear to be universal in that recent work in our laboratory with *S. aureus* (Buchanan et al. submitted) indicates that the MPD for this organism is strongly dependent of temperature, pH, and water activity.

Originally, it was hoped that the use of the square root transformation would stabilize the variance while permitting inclusion of the no-growth data. A ln transformation does not allow such data to be included since the ln of 0 is undefined. However, the trend toward

Table 6. Comparison of growth kinetics values for *Escherichia coli* cultured in various foods compared to predictions by response surface model^a.

Food	Predictions														
	Observation					Values assumed ^b					Growth kinetics				
	Temp (°C)	pH	LPD	GT	a_w	% NaCl	a_w	LPD	GT	Strain/serotype	Source				
Canned tuna	42	5.9	2.4	0.4	0.980	1.5	0.993	2.3	0.5	933/O157:H7	Current study				
						4.4	0.980	7.1	0.7						
	12	6.6	34.4	8.7	0.992	1.5	0.993	35.9	5.2	933/O157:H7		Current study			
Chicken broth	28	6.0	1.6	0.6	0.976	1.7	0.992	35.3	5.0	933/O157:H7	Current study				
						1.5	0.993	3.3	0.8						
UHT milk	19	6.5	6.3	1.9	0.985	5.4	0.976	9.1	1.2	933/O157:H7	Current study				
						0.5	0.997	8.1	1.3						
Raw mutton, blended	10	6.0 ^c	NR ^d	6.9	NR	3.1	0.985	12.9	2.3	SF/NR	Smith 1985				
	15			2.6		0.5		50.9	5.9						
	20			1.4				17.5	2.5						
	25			0.8				7.2	1.2						
	30			0.5				3.5	0.7						
	35			0.4				2.1	0.5						
	40			0.3				1.6	0.4						
Beef slices	10	6.0 ^e	NR	9.4	NR	0.5		1.4	0.3	Type 1/NR	Shaw and Nicol 1969				
	15			4.7				50.9	5.9						
	25			2.4				17.5	2.5						
	30			1.2				3.5	0.7						
	37			0.4				2.1	0.5						
Tryptic soy broth	7.3	7.3	≤2.0	0.4	NR	0.5		1.3	0.3	932, CL8, 933, EC2040, EC505B /O157:H7	Glass et al. 1992				
			≤2.0	0.4		1.5		1.8	0.4						
			2.0	0.5		2.5		2.9	0.5						
			7.0	1.6		4.5		9.0	0.6						
			≤2.0	0.4		0.5		1.3	0.3						
			≤2.0	0.4		0.5		1.3	0.3						
			≤2.0	0.4		0.5		1.5	0.3						
			2.3	0.5		0.5		1.9	0.4						
			3.3	0.5		0.5		2.6	0.5						
			4.7	0.8		0.5		4.0	0.7						

^aAerobic conditions assumed. Predicted values calculated using quadratic/in transformation models (Table 2).
^bThe NaCl values employed for solving the methods were based on: (1) assuming a concentration appropriate for the product; and (2) increasing the level to the calculated concentration that would produce the experimentally observed a_w . Where possible, the assumed value for NaCl content was estimated from the product label.
^cAssumed value; reported range was 5.7 to 6.3.
^dNot reported.
^eAssumed value.

under estimation of the potential for growth by the models based on the square root transformation in the specific range of most concern to food microbiologists favoured the use of the models based on a ln transformation. Because the no growth data were excluded during the development of ln-based models, this yielded models that were more conservative in their predictions. As with any regression based model, it is important to emphasize that the models should not be used to predict responses beyond the limits for which experimental data were collected to generate the models.

The limited quantitative data available on the growth kinetics of *E. coli*, particularly serovar O157:H7, precluded a detailed evaluation of how well microbiological medium-based models predict the micro-organism's behavior in food systems. However, the food data available, as well as past experiences with response surface models for other foodborne pathogens (Gibson et al. 1988, Buchanan and Phillips 1990, Palumbo et al. 1991, 1992, Zaika et al. 1992), suggest that the current models will be applicable to a range of foods. Further research will be needed to clarify the use of estimated NaCl concentration versus observed water activities (Table 6) for estimating the growth of *E. coli* in foods. A possible explanation for the improved predictions using assumed NaCl levels instead of observed water activity is that the foods in question (i.e. chicken broth, tunafish and UHT milk) contain additional humectants that the pathogen tolerated better than NaCl.

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The current models are based on analyzing the Gompertz *B* and *M* terms, and then using these values to calculate LPD and GT/EGR. Recently, several improvements in the Gompertz equation have been suggested (Garthright 1991, Zwietering et al. 1990). Garthright (1991) recommended that it would be better to directly model the experimental values for LPD and GT/EGR. This was not done in the current study so that the models developed for *E. coli* would be consistent in approach with those developed for other foodborne pathogens. All of the growth kinetics data for the various pathogens that have been generated in our laboratory is currently being reanalyzed to develop alternate models, and test the hypothesis of Garthright (1991). Initial analyses suggest that differences are minimal if a sufficiently large database is the basis for the response surface models.

In summary, the current study provides response surface models that can provide reasonable estimates for the effects of incubation temperature, initial pH, and sodium chloride content on the aerobic and anaerobic growth of *E. coli* O157:H7. These models have been incorporated into the latest version of the MFS Pathogen Modeling Program (version 3.1) which is available upon request. Further experimentation is currently underway to expand the *E. coli* models to include sodium nitrite as a fifth variable.

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