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Predictive models for growth of *Salmonella typhimurium* DT104 from low and high initial density on ground chicken with a natural microflora ☆

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Abstract

A single strain (ATCC 700408) of *Salmonella typhimurium* DT104 was used to investigate and model growth from a low ($1.12\log_{10}$ mpn g^{-1}) and high ($3.7\log_{10}$ cfu g^{-1}) initial density on ground chicken with a natural microflora. Kinetic data for growth of the pathogen on ground chicken were fit to a primary model to determine lag time (λ), maximum specific growth rate (μ) and maximum population density (N_{max}). Secondary models for λ, μ and N_{max}, as a function of temperature (10–40 °C), were developed and compared among initial densities. Variation of pathogen growth among replicates ($n = 4$ or 5) was higher at 10–18 °C than at 22–40 °C and was higher for N_{max} than λ and μ. Prediction problems were observed when secondary models developed with one initial density were used to predict λ, μ and N_{max} from the other initial density, especially at 10–18 °C and for N_{max}. These results indicated that variation of growth among replicate challenge studies and initial density are important factors to consider when developing predictive models for growth of *S. typhimurium* DT104 on ground chicken with a natural microflora.
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Keywords: *Salmonella typhimurium* DT104; Predictive models; Ground chicken; Growth

1. Introduction

Mathematical models that predict growth of pathogens on food are usually developed with sterile broth because enumeration of pathogens in food with natural microflora is difficult (McClure et al., 1994). However, models developed with sterile broth do not always provide reliable predictions of pathogen growth on non-sterile and non-homogeneous food (Ross, 1996). Thus, there is a need to develop models for growth of pathogens on food with natural microflora.

One approach to modeling pathogen growth on food with natural microflora is to use a strain with a physiological characteristic, such as antibiotic resistance, fluorescence or bioluminescence, that can distinguish it from other microorganisms (Jansson, 2003). However, marker strains that are generated by mutation, selection or genetic engineering must be validated before development of models because sometimes they display growth that is different from the parent strain (Oscar, 2003; Rang et al., 2003; Oscar et al., 2006).

Widespread use of antibiotics in animal food production has raised concern about emergence of antibiotic-resistant bacteria and their impact on public health (Snary et al., 2004). Some pathogen strains are resistant to multiple antibiotics and have caused outbreaks (Akkina et al., 1999). For example, two multiple antibiotic-resistant strains of *Salmonella typhimurium* definitive phage type 104 (DT104) were responsible for four outbreaks in three states that involved three veterinary clinics, one animal shelter and 45 cases of salmonellosis among employees, clients and companion animals (Wright et al., 2005).

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Although multiple antibiotic-resistant strains of pathogens that occur in nature are alarming, predictive microbiologists could take advantage of these strains to develop better models. In fact, multiple antibiotic-resistant strains generated by selection in the laboratory have been used to investigate and model growth of *Salmonella* in minced beef (Mackey and Kerridge, 1988). However, use of multiple antibiotic-resistant strains that occur in nature, such as *S. typhimurium* DT104, to develop models for growth of pathogens on food with natural microflora has not been reported.

Poultry is a primary vector of *Salmonella* transmission to humans (Bryan and Doyle, 1995). The US Department of Agriculture reported that 45% of retail samples of ground chicken contain *Salmonella* and that the average level of contamination is $0.1\log_{10}\text{mpn g}^{-1}$ (Anonymous, 1996). Predictive models are normally developed with a high initial density ($3–6\log_{10}\text{cfu ml}^{-1}$) of the test pathogen. However, Oscar (2005a) reported that a predictive model developed with a high initial density ($4.8\log_{10}\text{cfu g}^{-1}$) of *Salmonella typhimurium* did not provide acceptable predictions of growth from a lower initial density ($0.8\log_{10}\text{mpn g}^{-1}$) on sterile chicken. Consequently, the objective of the current study was to investigate and model growth of *S. typhimurium* DT104 from low ($1.12\log_{10}\text{mpn g}^{-1}$) and high ($3.7\log_{10}\text{cfu g}^{-1}$) initial density on ground chicken with a natural microflora.

2. Materials and methods

2.1. Bacterial strain and preparation

Stock cultures of *Salmonella typhimurium* DT104 (ATCC 700408, American Type Culture Collection, Manassas, VA, USA) were maintained at −70 °C in brain heart infusion broth (Becton Dickinson, Sparks, MD, USA) that contained 15% glycerol (Sigma Chemical Company, St. Louis, MO, USA). On a weekly basis, pathogen cells for inoculation of ground chicken were prepared by adding stock culture (5 μl) to sterile brain heart infusion broth (5 ml) in an Erlenmeyer flask (25-ml) sealed with a foam plug followed by incubation at 30 °C and 150 orbits per minute (opm) for 23 h. Immediately before inoculation, cultures were serially diluted in sterile buffered peptone water (Becton Dickinson).

2.2. Challenge studies with ground chicken

Boneless chicken breast meat was purchased weekly from local retail outlets. The chicken was ground through an χ inch plate using an electric meat grinder (The Sausage Maker, Buffalo, NY, USA) and divided into 1-g portions for challenge studies.

Diluted culture (5 μl) of *S. typhimurium* DT104 was inoculated onto ground chicken portions for an average initial pathogen density of $1.12\log_{10}$ most probable number (mpn) g^{-1} or $3.7\log_{10}$ colony forming units (cfu) g^{-1}. Inoculated samples were incubated at 10, 12, 14, 18, 22, 26, 30, 34 or 40 °C. At selected times of incubation, a 1-g portion was homogenized (model 80 stomacher blender, Seward, London, UK) in sterile buffered peptone water (9 ml) for pathogen enumeration. Four growth curves were conducted per temperature for the low initial density and five growth curves per temperature were conducted for the high initial density. Two growth curves at different temperatures were conducted per week so that different batches of ground chicken and inoculation culture were used for each combination of initial density and temperature.

2.3. Pathogen enumeration

Homogenized samples of ground chicken that were inoculated with *S. typhimurium* DT104 were serially diluted in sterile buffered peptone water and spiral plated (Whitley Automatic Spiral Plater, Microbiology International, Frederick, MD, USA) onto xylose lysine (XL) agar medium (Becton Dickson) that contained 25 mM *N*-[2-hydroxyethyl]-piperazine-*N*′-[2-ethanesulfonic acid] or HEPES (H) and $25\,\mu\text{g ml}^{-1}$ of the following antibiotics from Sigma: chloramphenicol (C), ampicillin (A), tetracycline (T) and streptomycin (S); hereafter, referred to as XLH-CATS. The buffering agent HEPES was included to prevent formation of yellow acid zones around the black colonies of *S. typhimurium* DT104 that formed on XLH-CATS. The yellow acid zones interfered with automated counting of colonies (ProtoCol Automated Colony Counter, Microbiology International), which was performed after incubation of spiral plates for 24 h at 38 °C.

Salmonella typhimurium DT104 was also enumerated using a 3×4 most probable number method with a detection range from 0 to $3.28\log_{10}\text{mpn g}^{-1}$. The mpn tubes were prepared by serial dilution of homogenized samples in sterile buffered peptone water. After incubation of mpn tubes for 24 h at 38 °C, a 2 μl sample from the mpn tubes was spot inoculated onto XLH-CATS. After 24 h of incubation at 38 °C, the mpn assay on XLH-CATS was read and recorded. The method of Thomas (1942) was used to calculate the mpn results.

2.4. Primary modeling

Viable count ($\log_{10}\text{cfu g}^{-1}$) and mpn data ($\log_{10}\text{mpn g}^{-1}$) were graphed as a function of time and were fit (Prism version 4.0, GraphPad Software, San Diego, CA, USA) to the modified Gompertz model (Zwietering et al., 1990):

$$N(t) = N_0 + (N_{\max} - N_0) \times [\exp(-\exp((2.718\,\mu/(N_{\max} - N_0))(\lambda - t) + 1))],$$

where $N(t)$ is pathogen density ($\log_{10}$ mpn or cfu g^{-1}) at time t (h), N_0 is initial pathogen density ($\log_{10}$ mpn or cfu g^{-1}), $N_{\max}$ is maximum population density ($\log_{10}$ mpn

or cfu g^{-1}), μ is maximum specific growth rate (h^{-1}) and λ is lag time (h).

2.5. Secondary modeling

Natural logarithm transformations (ln) of λ and μ from primary modeling were graphed as a function of temperature (T, °C) and were fit (Prism software) to a quadratic polynomial model:

$$\ln \lambda \quad \text{or} \quad \ln \mu = a + bT + cT^2,$$

where a, b and c are regression coefficients.

Natural logarithm transformations of N_{max} were graphed as a function of temperature and were fit to an asymptote model (Wijtzes et al., 1995):

$$\ln N_{max} = [a(T - T_{min})]/[(T - T_{sub\ min})],$$

where a is a regression coefficient, T_{min} the minimum growth temperature (°C) and $T_{sub\,min}$ the temperature (°C) just below T_{min}.

2.6. Evaluation of model performance

Goodness-of-fit of primary and secondary models was evaluated using the coefficient of determination (R^2). Prediction bias and accuracy of secondary models for data used in model development (dependent data) and for data not used in model development (independent data) were evaluated using the acceptable prediction zone method

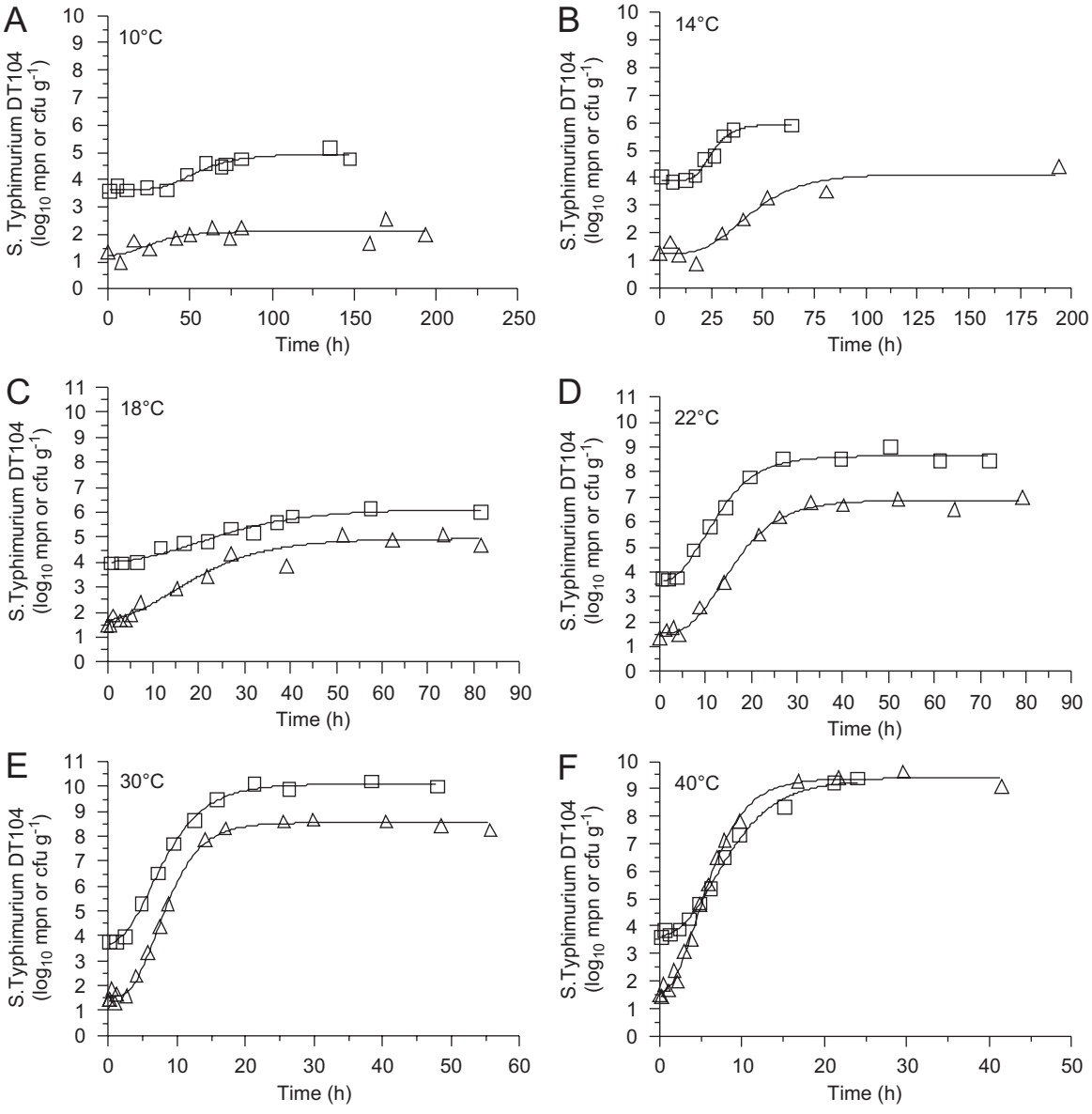

Fig. 1. Representative primary model fits for growth of *Salmonella typhimurium* DT104 from low (Δ) or high (□) initial density on ground chicken incubated at (A) 10 °C, (B) 14 °C, (C) 18 °C, (D) 22 °C, (E) 30 °C or (F) 40 °C.

(Oscar, 2005a, b). Prediction errors (PE) for individual prediction cases were calculated:

$$\text{PE for } \lambda\ (\%) = [(\text{predicted} - \text{observed})/\text{predicted}] \times 100,$$

$$\text{PE for } \mu\ (\%) = [(\text{observed} - \text{predicted})/\text{predicted}] \times 100,$$

$$\text{PE for } N_{\max}\ (\log_{10}\text{mpn or cfu g}^{-1}) = (\text{observed} - \text{predicted}),$$

such that PE less than zero represented fail-safe predictions and PE greater than zero represented fail-dangerous predictions. Model performance was quantified using the percentage of prediction errors (%PE) in an acceptable prediction zone that was twice as wide in the fail-safe direction as in the fail-dangerous direction. Widths of acceptable prediction zones are based on an assessment of experimental error associated with determining individual growth parameters. Width of the acceptable prediction zone for evaluation of model performance were −60% to 30% for λ, −30% to 15% for μ and −1 to 0.5 $\log_{10}$ mpn or cfu g^{-1} for $N_{\max}$.

Presence of regional prediction problems was evaluated by visual examination of PE plots and by calculating %PE

Table 1
Statistical summary of primary modeling for growth of *Salmonella typhimurium* DT104 on ground chicken as a function of initial density and temperature: coefficient of determination (R^2)

Initial density	Temperature (°C)	*n*	Mean	Standard deviation	Coefficient of variation	Minimum	Maximum
1.12 $\log_{10}$ mpn g^{-1}	10	3	0.7780	0.1411	18.1	0.6182	0.8855
	12	4	0.9373	0.0315	3.4	0.9019	0.9781
	14	4	0.9694	0.0226	2.3	0.9391	0.9876
	18	4	0.9652	0.0117	1.2	0.9558	0.9814
	22	4	0.9922	0.0038	0.4	0.9885	0.9975
	26	4	0.9929	0.0043	0.4	0.9868	0.9967
	30	4	0.9949	0.0035	0.4	0.9896	0.9967
	34	4	0.9897	0.0039	0.4	0.9861	0.9951
	40	4	0.9964	0.0017	0.2	0.9947	0.9982
3.70 $\log_{10}$ cfu g^{-1}	10	5	0.9493	0.0418	4.4	0.8881	0.9936
	12	5	0.9207	0.0751	8.2	0.8243	0.9961
	14	5	0.9875	0.0066	0.7	0.9785	0.9970
	18	5	0.9894	0.0160	1.6	0.9611	0.9990
	22	5	0.9905	0.0125	1.3	0.9685	0.9990
	26	5	0.9902	0.0084	0.8	0.9761	0.9974
	30	5	0.9951	0.0035	0.4	0.9906	0.9980
	34	5	0.9922	0.0098	1.0	0.9747	0.9982
	40	5	0.9955	0.0006	0.1	0.9948	0.9963

Table 2
Statistical summary of primary modeling for growth of *Salmonella typhimurium* DT104 on ground chicken as a function of initial density and temperature: lag time (h)

Initial density	Temperature (°C)	*n*	Mean	Standard deviation	Coefficient of variation	Minimum	Maximum
1.12 $\log_{10}$ mpn g^{-1}	10	3	21.90	12.61	57.6	8.15	32.91
	12	4	22.26	9.34	41.9	11.40	32.09
	14	4	11.41	8.94	78.3	2.58	21.35
	18	4	4.98	2.12	42.7	3.00	7.55
	22	4	5.65	1.52	26.9	4.23	7.39
	26	4	3.50	2.15	61.6	1.14	6.34
	30	4	2.85	1.08	37.8	1.30	3.60
	34	4	2.25	0.34	14.9	1.93	2.71
	40	4	1.91	0.35	18.5	1.52	2.37
3.70 $\log_{10}$ cfu g^{-1}	10	5	36.18	5.17	14.3	29.41	42.12
	12	5	27.61	15.99	57.9	19.45	56.14
	14	5	27.22	17.21	63.2	11.40	46.74
	18	5	12.73	6.68	52.5	6.70	22.51
	22	5	4.80	1.23	25.6	3.91	6.95
	26	5	2.46	0.87	35.3	1.46	3.85
	30	5	1.99	0.57	28.6	1.47	2.85
	34	5	1.39	0.63	45.6	0.49	2.10
	40	5	1.66	0.57	34.5	1.12	2.53

for low (10–18 °C) and high (22–40 °C) temperatures. For clarity of presentation, PE greater than 100% or less than −100% were graphed as 100% and −100%, respectively.

3. Results

3.1. Primary modeling

Growth of *S. typhimurium* DT104 from low and high initial density on ground chicken followed a sigmoid pattern (Fig. 1). Goodness-of-fit of the primary model to the mpn and cfu data was affected by initial density and temperature (Table 1). Lower R^2 values were observed at lower temperatures (10–18 °C) than at higher temperatures (22–40 °C). In addition, lower R^2 values were observed for growth from the lower initial density than the higher initial density at lower but not higher temperatures. Goodness-of-fit of the primary model was lowest and most variable among replicates for the data collected at 10 and 12 °C. Regardless of initial density, R^2 values were high (>0.96)

Table 3
Statistical summary of primary modeling for growth of *Salmonella typhimurium* DT104 on ground chicken as a function of initial density and temperature: maximum specific growth rate (h^{-1})

Initial density	Temperature (°C)	*n*	Mean	Standard deviation	Coefficient of variation	Minimum	Maximum
1.12 $\log_{10}$ mpn g^{-1}	10	4	0.026	0.019	74.6	0.000	0.043
	12	4	0.061	0.016	25.7	0.039	0.075
	14	4	0.071	0.021	29.1	0.056	0.102
	18	4	0.111	0.012	10.9	0.101	0.127
	22	4	0.305	0.020	6.6	0.287	0.334
	26	4	0.465	0.097	21.0	0.391	0.606
	30	4	0.682	0.083	12.1	0.565	0.758
	34	4	0.831	0.026	3.1	0.801	0.862
	40	4	1.025	0.091	8.9	0.949	1.156
3.70 $\log_{10}$ cfu g^{-1}	10	5	0.031	0.014	45.9	0.018	0.051
	12	5	0.046	0.019	41.6	0.019	0.067
	14	5	0.077	0.034	43.6	0.035	0.119
	18	5	0.120	0.054	44.7	0.061	0.186
	22	5	0.270	0.059	21.9	0.187	0.333
	26	5	0.425	0.062	14.6	0.374	0.513
	30	5	0.604	0.087	14.4	0.555	0.759
	34	5	0.723	0.077	10.7	0.647	0.832
	40	5	0.779	0.180	23.1	0.517	0.947

Table 4
Statistical summary of primary modeling for growth of *Salmonella typhimurium* DT104 on ground chicken as a function of initial density and temperature: maximum population density ($\log_{10}$ mpn or cfu g^{-1})

Initial density	Temperature (°C)	*n*	Mean	Standard deviation	Coefficient of variation[a]	Minimum	Maximum
1.12 $\log_{10}$ mpn g^{-1}	10	4	1.83	0.19	50.4	1.67	2.10
	12	4	3.62	1.04	171.6	2.70	4.93
	14	4	6.01	1.63	194.0	4.12	8.04
	18	4	5.51	0.84	178.4	4.93	6.71
	22	4	7.31	0.82	136.9	6.43	8.25
	26	4	7.84	0.67	89.3	6.95	8.38
	30	4	8.86	0.99	167.2	7.73	10.07
	34	4	9.27	0.55	90.9	8.52	9.82
	40	4	9.55	0.20	46.7	9.38	9.79
3.70 $\log_{10}$ cfu g^{-1}	10	5	5.02	0.45	109.9	4.61	5.67
	12	5	5.89	1.28	221.4	4.94	8.12
	14	5	7.28	1.19	154.0	5.85	8.68
	18	5	8.43	1.60	148.9	6.12	10.04
	22	5	9.04	0.86	126.9	8.21	9.97
	26	5	9.65	0.46	60.1	8.87	9.99
	30	5	9.52	0.73	84.5	8.58	10.09
	34	5	9.75	0.72	64.2	8.49	10.20
	40	5	9.69	0.41	71.2	9.19	10.09

[a]The coefficient of variation was calculated using N_{max} values expressed as mpn or cfu g^{-1} and not as $\log_{10}$ mpn or cfu g^{-1} so as to provide a better assessment of the variation of N_{max} among replicate challenge studies.

and variation low (<1.5%) for primary model fits to data obtained at higher temperatures.

Variation of λ (Table 2) and μ (Table 3) among replicate challenge studies was, in most cases, higher at 10–18 °C than at 22–40 °C, whereas variation of N_{max} (Table 4) among replicate challenge studies was, in most cases, higher at 12–22 °C than at 10 °C and 26–40 °C. Among growth parameters, N_{max} showed the highest variation among replicate challenge studies followed by λ and then μ. These results demonstrated that variation of λ, μ and N_{max} among replicate challenge studies was not constant as a function of temperature.

3.2. Secondary modeling

Because variation of growth parameters was not constant as a function of temperature, secondary models were developed with natural logarithm transformations of λ, μ and N_{max} to stabilize model variance. The R^2 values for the secondary model fits (Table 5) indicated that the models had high goodness-of-fit for mean values of λ, μ and N_{max} but lower goodness-of-fit for replicate values of the growth parameters. The difference in R^2 values between replicate and mean values was highest for λ, lowest for μ, and intermediate for N_{max}.

Graphs of secondary model fits to the growth parameter data indicated that initial density altered growth of *S. typhimurium* DT104 on ground chicken with N_{max} being affected more than λ and μ (Fig. 2). The effect of initial density on N_{max} was greatest at 10 °C and then decreased in magnitude as temperature increased until there was no effect of initial density on N_{max} at 40 °C (Fig. 2c). Smaller effects of initial density on λ and μ were observed. Growth from a lower initial density resulted in shorter λ at 10–18 °C, similar λ at 22 and 26 °C and longer λ at 30–40 °C (Fig. 2a). Maximum specific growth rate was slightly higher at all temperatures when growth occurred from the lower initial density with the largest difference in μ observed at 40 °C (Fig. 2b).

3.3. Evaluation of secondary model performance

Table 6 shows results of the acceptable prediction zone analyses for the low initial density and high initial density models for replicate and mean values of λ, μ and N_{max}. Results for dependent data indicate how well the model predicted the growth parameter data used in model development (goodness-of-fit). Results for independent data indicate how well the model predicted the growth parameter data collected with the other initial density (extrapolation). Plots of PE for replicate (Fig. 3) and mean (Fig. 4) data as well as %PE for lower (10–18 °C) and higher (22–40 °C) temperatures (Table 6) evaluated the models for regional prediction problems, which were observed.

3.3.1. Dependent data

Model performance (goodness-of-fit) was better for higher (22–40 °C) temperatures than lower (10–18 °C)

Table 5
Statistical summary of secondary modeling for lag time (λ), maximum specific growth rate (μ) and maximum population density (N_{max}) of *Salmonella typhimurium* DT104 on ground chicken: comparison of fits to replicate and mean values

Secondary model	Initial density	Model parameter	Replicate			Mean		
			Best-fit value	±95% Confidence Interval	R^2	Best-fit value	±95% Confidence Interval	R^2
Quadratic Polynomial $\ln\lambda$	1.12 $\log_{10}$ mpn g^{-1}	*a*	4.856	1.257	0.7003	5.046	1.158	0.9587
		b	−0.2165	0.1138		−0.2188	0.1065	
		c	0.00282	0.002283		0.00276	0.002155	
	3.70 $\log_{10}$ cfu g^{-1}	*a*	6.521	0.8435	0.8971	6.606	1.328	0.9735
		b	−0.3140	0.07760		−0.3142	0.1221	
		c	0.003961	0.001570		0.003947	0.002471	
Quadratic Polynomial $\ln\mu$	1.12 $\log_{10}$ mpn g^{-1}	*a*	−5.853	0.4985	0.9703	−6.159	0.936	0.9867
		b	0.2776	0.0452		0.3019	0.0860	
		c	−0.003248	−0.000906		−0.003682	0.001742	
	3.70 $\log_{10}$ cfu g^{-1}	*a*	−6.325	0.6655	0.9294	−6.131	0.4620	0.9963
		b	0.3131	0.06125		0.3025	0.04250	
		c	−0.004026	0.00124		−0.003874	0.0008600	
Asymptote $\ln N_{max}$	1.12 $\log_{10}$ mpn g^{-1}	*a*	2.396	0.1585	0.9066	2.378	0.2395	0.9682
		T_{min}	9.175	0.4910		9.223	0.7265	
		$T_{sub\ min}$	6.740	1.445		6.979	2.113	
	3.70 $\log_{10}$ cfu g^{-1}	*a*	2.433	0.1350	0.7988	2.424	0.1065	0.9830
		T_{min}	6.606	2.214		6.813	1.733	
		$T_{sub\ min}$	4.799	3.425		5.150	2.652	

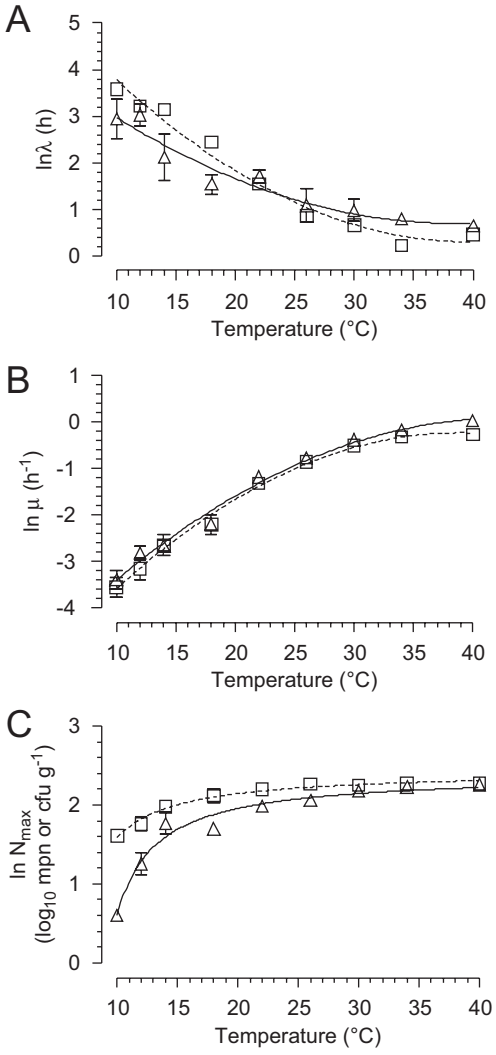

Fig. 2. Secondary model fits to natural logarithm transformations (ln) of (A) lag time (λ), (B) maximum specific growth rate (μ), and (C) maximum population density (N_{max}) of *Salmonella typhimurium* DT104 from low (Δ) or high ($\square$) initial density on ground chicken. Symbols are means ± standard errors of the mean.

temperatures and for mean than replicate data (Table 6). The average %PE value for higher temperatures was 80% for replicate data and 95% for mean data as compared to 46% for replicate data and 83% for mean data at lower temperatures. The PE plots in Fig. 3 for dependent data demonstrate that at lower temperatures, more PE were outside the acceptable prediction zones for λ, μ and N_{max} than at higher temperatures.

When dependent data for all temperatures (10–40 °C) were evaluated together, the average %PE for replicate data was 65% as compared to 90% for mean data. In Fig. 4, PE plots for mean data show that very few PE for dependent data were outside the acceptable prediction zones for the growth parameters. Similar to the R^2 values, the acceptable prediction zone analyses indicated high goodness-of-fit of the models to mean data and lower goodness-of-fit to replicate data.

3.3.2. Independent data

The secondary models for low and high initial density were evaluated for the ability to predict the growth parameter values obtained with the other initial density (independent data). Similar to dependent data, model performance (extrapolation) was better for higher (22–40 °C) temperatures than lower (10–18 °C) temperatures and for mean than replicate data (Table 6). The average %PE for higher temperatures were 51% for replicate data and 63% for mean data as compared to 29% for replicate data and 38% for mean data at lower temperatures. The PE plots for independent data in Fig. 3 demonstrate that the number of PE outside the acceptable prediction zone decreased as temperature increased and that more PE for N_{max} were outside the acceptable prediction zone than for λ and μ, especially at lower temperatures. In fact, the average %PE for higher temperatures were 64% for λ, 51% for μ and 38% for N_{max} as compared to 44% for λ, 38% for μ and 6% for N_{max} at lower temperatures.

When independent data for all temperatures (10–40 °C) were evaluated together, the average %PE for replicate data was 41% as compared to 52% for mean data. The average %PE for lower temperatures were 25% for λ, 88% for μ and 0% for N_{max} as compared to 90% for λ, 70% for μ and 30% for N_{max} at higher temperatures. These results and the PE plots for mean data in Fig. 4 for independent data demonstrate that initial density had the largest effect on N_{max} followed by λ at lower temperatures and then μ, which was not greatly affected by initial density at any temperature investigated.

4. Discussion

4.1. Variation of growth among replicate challenge studies

Variation of λ among replicate challenge studies was significant and could have resulted from variation of the previous history of the pathogen (Buchanan and Klawitter, 1991; Hudson, 1993). However, in the present study, growth conditions of the inoculation culture were tightly controlled. In fact, the same batch of stock culture and the same batch of sterile brain heart infusion broth were used to prepare all inoculation cultures and standard growth conditions for the inoculation culture were used throughout the study. Moreover, previous studies (Oscar, 1999a–c) indicate that subsequent growth of *Salmonella* in sterile broth or on sterile chicken is not affected by changes in previous pH (5.7–8.6), previous temperature (16–34 °C) or previous sodium chloride level (0.5–4.5%) of the inoculation culture. Thus, variation of previous history of the pathogen was not a likely explanation for the observed variation of λ of *S. typhimurium* DT104 among replicate challenge studies.

Similar to results of this study, growth of *Salmonella* in enrichment cultures of food samples with natural

Table 6
Evaluation of performance of secondary models for predicting lag time (λ), maximum specific growth rate (μ) and maximum population density (N_{max}) of *Salmonella typhimurium* DT104 on ground chicken as a function of initial density and temperature: percentage of prediction errors (%PE) in the acceptable prediction zone for replicate and mean values of the growth parameters

Secondary model	Initial density	Dataset[a]	Replicate			Mean		
			10–18 °C	22–40 °C	10–40 °C	10–18 °C	22–40 °C	10–40 °C
λ	1.12 $\log_{10}$ mpn g^{-1}	Dependent	38	80	61	75	100	89
		Independent	38	65	53	25	100	67
	3.70 $\log_{10}$ cfu g^{-1}	Dependent	65	88	78	100	100	100
		Independent	50	64	58	25	80	56
μ	1.12 $\log_{10}$ mpn g^{-1}	Dependent	44	85	67	75	100	89
		Independent	56	30	42	75	40	56
	3.70 $\log_{10}$ cfu g^{-1}	Dependent	25	64	47	100	100	100
		Independent	20	72	49	100	100	100
N_{max}	1.12 $\log_{10}$ mpn g^{-1}	Dependent	56	60	58	50	100	78
		Independent	6	55	33	0	60	30
	3.70 $\log_{10}$ cfu g^{-1}	Dependent	50	100	78	100	100	100
		Independent	5	20	13	0	0	0

[a]Dependent data were growth parameter data used in model development, whereas independent data were data for growth parameters from the other initial density. Thus, %PE values for dependent data evaluate goodness-of-fit of the model, whereas %PE values for independent data evaluate how well the model predicts growth from the other initial density.

microflora is variable among batches (Rhodes et al., 1985) with N_{max} of *Salmonella* in buffered peptone water ranging from 3 to 7 $\log_{10}$ cfu ml^{-1} (Beckers et al., 1987). This variation in growth of *Salmonella* among food sample enrichments is attributed to variations in numbers and types of native microorganisms (Beckers et al., 1987). In a comparison of enrichments with and without natural microflora, Stecchini et al. (1988) found that the natural microflora reduced growth rate and total growth of *Salmonella*. Thus, variation of natural microflora among batches of ground chicken is a potential explanation for the observed variation of λ, μ and N_{max} among replicate challenge studies in this research.

The natural microflora on freshly processed chickens has been characterized and includes gram-positive rods, Enterobacteriaceae, Micrococci, Streptococci, Flavobacteria, Lactobacilli, *Aeromonas*, *Moraxella*, *Acinetobacter*, *Brochothrix thermosphacta* and *Pseudomonas* (Schmitt et al., 1988). Post-processing storage conditions determine which species and strains predominate and which genes are expressed by the native microflora. For example, at 30 °C, poultry strain F-21 of *Pseudomonas fluorescens* produces a pigment that inhibits growth of *Salmonella*, whereas, at 37 °C, this strain does not produce the pigment or inhibit growth of *Salmonella* (Oblinger and Kraft, 1970). In the current study, variation of *S. typhimurium* DT104 growth among replicate challenge studies was higher at 10–18 °C than at 22–40 °C. It is interesting to speculate that the higher variation of *S. typhimurium* DT104 growth at lower temperatures could be caused by batch-to-batch variation in the levels or activity of specific inhibitory strains in the natural microflora of ground chicken.

4.2. Effect of initial density on pathogen growth

In sterile broth, growth of food pathogens is usually independent of initial density and N_{max} is usually not greatly affected by growth conditions (Buchanan and Phillips, 1990; Buchanan et al., 1993). Consequently, models developed with one initial density can be extrapolated to other initial densities and provide reliable predictions of λ, μ and N_{max}. A different situation was encountered in the present study for growth of *S. typhimurium* DT104 on ground chicken with a natural microflora. When models developed with one initial density were used to predict growth parameters obtained with the other initial density, unacceptable predictions of λ, μ and N_{max} were obtained. These results indicate that in contrast to modeling growth in sterile broth, initial density is an important factor to consider when modeling growth of *S. typhimurium* DT104 on ground chicken with a natural microflora. This conclusion is consistent with previous results with sterile chicken (Oscar, 2005a) but does not agree with findings in minced beef incubated at 10–35 °C where initial density (1.6 versus 4 $\log_{10}$ cfu g^{-1}) did not alter λ, μ or N_{max} of a three strain cocktail of *Salmonella* (Mackey and Kerridge, 1988).

4.3. Evaluation of model performance

The prediction bias (B_f) and accuracy (A_f) factors are the most widely used method for evaluating performance of predictive models (Ross, 1996). However, when compared to the acceptable prediction zone method, B_f and A_f were found less effective at detecting prediction problems in published growth models for *Salmonella* and *Escherichia*

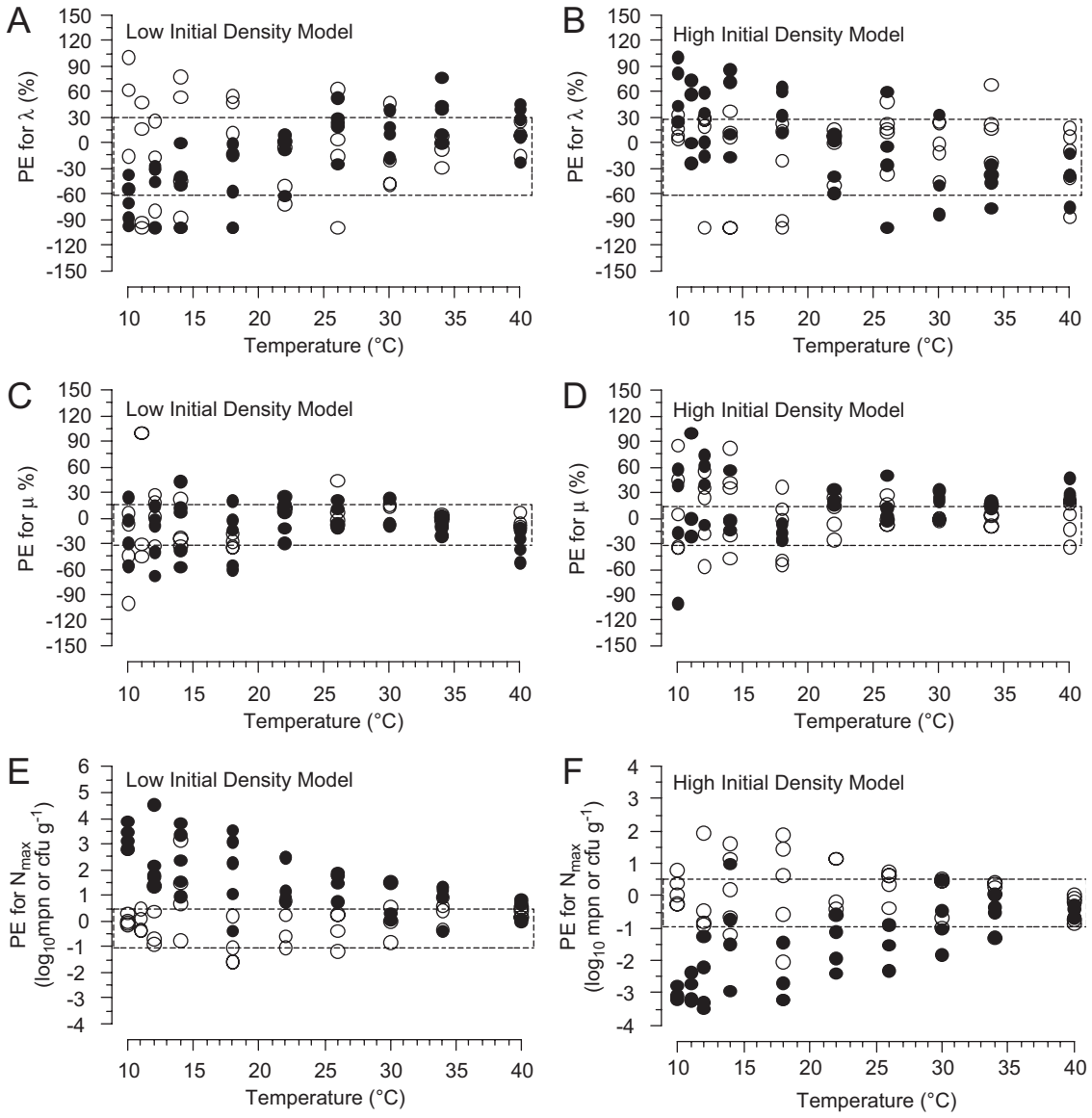

Fig. 3. Acceptable prediction zone analysis of prediction errors (PE) for dependent (○) and independent (●) data (replicate) for (A) the low initial density model for lag time (λ), (B) the high initial density model for λ, (C) the low initial density model for maximum specific growth rate (μ), (D) the high initial density model for μ, (E) the low initial density model for maximum population density (N_{max}) and (F) the high initial density model for N_{max}.

coli O157:H7 (Oscar, 2005b). Consequently, the acceptable prediction zone method rather than B_f and A_f were used to evaluate model performance in this study.

The criterion for acceptable performance of a model in the acceptable prediction zone method is that 70% or more of the predictions errors should be in the acceptable prediction zone for the growth parameter (Oscar, 2005b). The acceptable prediction zone is wider in the fail-safe direction than in the fail-dangerous direction because greater prediction error can be tolerated in the fail-safe direction when models are used to predict food safety (Ross et al., 2000). Different acceptable prediction zones are used for evaluating performance of secondary models for λ, μ and N_{max} because the experimental error for determining the individual growth parameters differs (Oscar, 2005a). In the current study, all secondary models for λ, μ and N_{max} had %PE of 70% or higher when mean values were evaluated. However, when replicate values were evaluated, only the high initial density models for λ and N_{max} had %PE of 70% or more. Failure of the other models to provide acceptable predictions of the growth parameters was primarily due to unacceptable performance at the lower incubation temperatures of 10–18 °C, where variation of growth parameters among replicates was highest. Including an independent variable in the model in addition to temperature that can help explain the variation of growth among replicates at lower temperatures might result in secondary models with acceptable performance for predicting replicate values of λ, μ and N_{max}. For example, adding an independent variable for the initial level of a general group (total aerobes), specific genus (*Pseudomonas*) or strain (*P. fluorescens* F-21) of the native

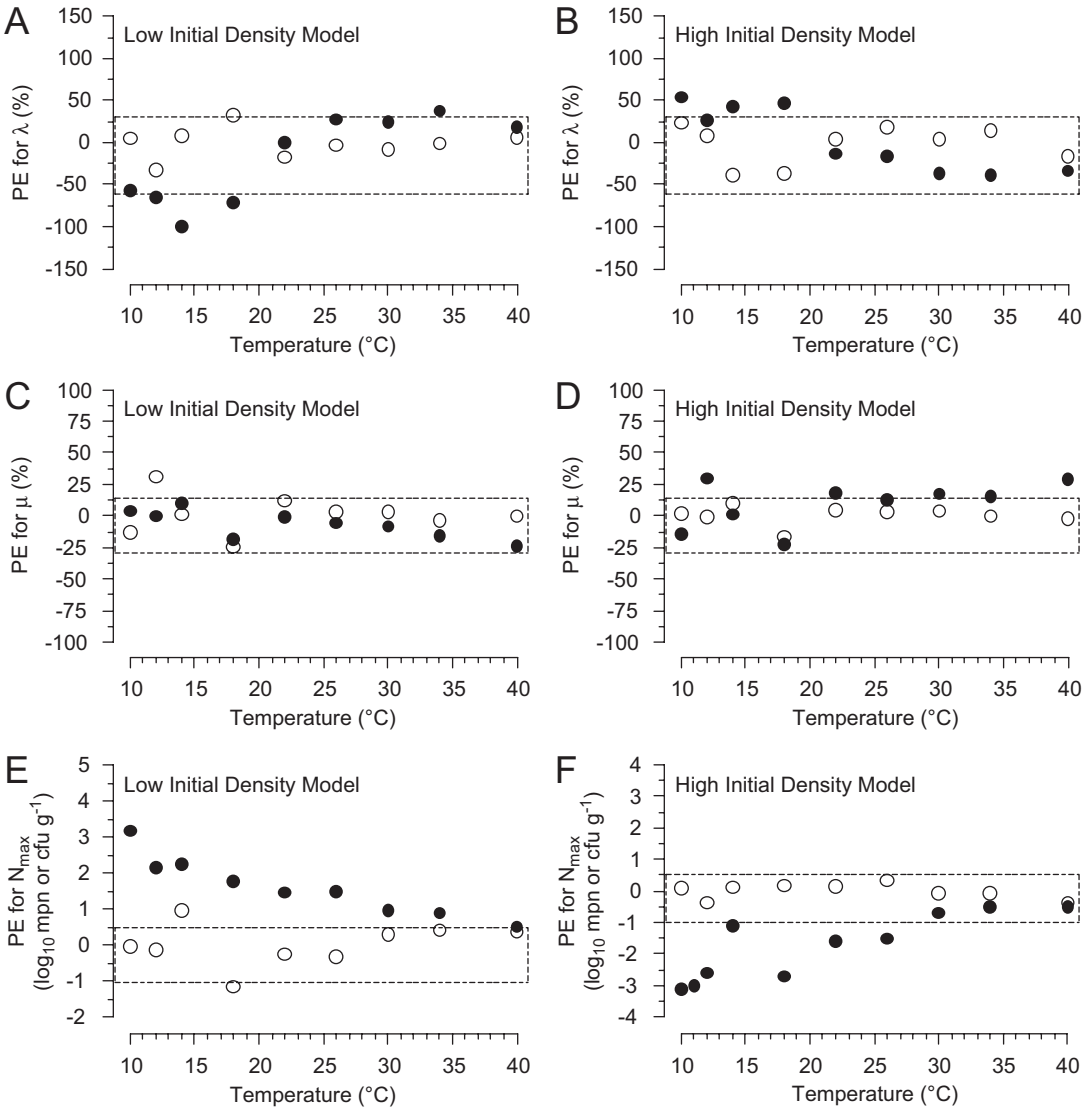

Fig. 4. Acceptable prediction zone analysis of prediction errors (PE) for dependent (○) and independent (●) data (mean) for (A) the low initial density model for lag time (λ), (B) the high initial density model for λ, (C) the low initial density model for maximum specific growth rate (μ), (D) the high initial density model for μ, (E) the low initial density model for maximum population density (N_{max}) and (F) the high initial density model for N_{max}.

microflora or activity of an anti-*Salmonella* compound produced by the native microflora, such as the inhibitory pigment of *P. fluorescens* F-21 (Oblinger and Kraft, 1970), might improve the ability of the secondary models to predict the variation of pathogen growth among replicate batches of ground chicken.

4.4. *S. typhimurium DT104 (ATCC 700408) as a surrogate for other Salmonella*

An accomplishment of the present study was the successful modeling of *Salmonella* growth on ground chicken with a natural microflora using a strain (*S. typhimurium* DT104 ATCC 700408) that occurs in nature. Modeling was accomplished by taking advantage of the strain's natural resistance to multiple antibiotics and its selective growth requirements. Agar plates with selective ingredients including multiple antibiotics were incubated at an optimal growth temperature (38 °C) for the pathogen and were observed to be free of native microflora throughout the study, which made it easy to enumerate the pathogen after inoculation onto ground chicken. A non-selective medium with multiple antibiotics was not used because it was previously demonstrated that such an approach did not suppress growth of native food microorganisms enough to allow enumeration of inoculated pathogens (Blackburn and Davies, 1994).

Although it is possible that indigenous *S. typhimurium* DT104 could be present in ground chicken and inflate mpn and cfu values, there was no evidence (outliers) of this in the current study. The use of a small portion size (1-g) and the reported low prevalence of *S. typhimurium* DT104 on chicken (Antunes et al., 2003) could account for these observations. In fact, in a separate study (Oscar,

unpublished data), when samples of chicken thigh skin (2.83 cm^2) were incubated in sterile buffered peptone water for 24 h at 38 °C followed by inoculation and incubation on XLH-CATS, zero of 100 samples were positive for *Salmonella*.

There are over 2000 serotypes and many more strains of *Salmonella*. Which serotype or strain to use in model development is an important consideration as variation of growth among *Salmonella* has been observed (Fehlhaber and Kruger, 1998; Oscar, 2000). Before selecting the strain of *S. typhimurium* DT104 used in this study to develop predictive models, its growth kinetics on sterile chicken at 10–40 °C were compared and found to be similar to those of *S. typhimurium* ATCC 14028 (Oscar, unpublished data). The latter strain has been used to model growth of *Salmonella* (Thayer et al., 1987; Oscar, 1999c) and has been shown to have similar growth kinetics as other strains of *Salmonella* from chicken (Oscar, 1998). Thus, the strain used in this study was considered an appropriate surrogate strain for modeling *Salmonella* growth on ground chicken with a native microflora.

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References

Akkina, J.E., Hougue, A.T., Angulo, R.J., Johnson, R., Petersen, K.E., Saini, P.K., Fedorka-Cray, P.J., Schlosser, W.E., 1999. Epidemiologic aspects, control, and importance of multiple-drug resistant *Salmonella typhimurium* DT104 in the United States. J. Am. Vet. Med. Assoc. 214, 790–798.

Anonymous, 1996. Nationwide Raw Ground Chicken Microbiological Survey. US Department of Agriculture, Food Safety and Inspection Service, May 1–8.

Antunes, P., Reu, C., Sousa, J.C., Peixe, L., Pestana, N., 2003. Incidence of *Salmonella* from poultry products and their susceptibility to antimicrobial agents. Int. J. Food Microbiol. 82, 97–103.

Beckers, H.J., Heide, J.V.D., Fenigsen-Narucka, U., Peters, R., 1987. Fate of salmonellas and competing flora in meat sample enrichments in buffered peptone water and in Muller–Kauffmann's tetrathionate medium. J. Appl. Bacteriol. 62, 97–104.

Blackburn, C.W., Davies, A.R., 1994. Development of antibiotic-resistant strains for the enumeration of food borne pathogenic bacteria in stored foods. Int. J. Food Microbiol. 24, 125–136.

Bryan, F.L., Doyle, M.P., 1995. Health risks and consequences of *Salmonella* and *Campylobacter jejuni* in raw poultry. J. Food Prot. 58, 326–344.

Buchanan, R.L., Bagi, L.K., Goins, R.V., Phillips, J.G., 1993. Response surface models for the growth kinetics of *E. coli* O157:H7. Food Microbiol. 10, 303–315.

Buchanan, R.L., Klawitter, L.A., 1991. Effect of temperature history on the growth of *Listeria monocytogenes* Scott A at refrigeration temperature. Int. J. Food Microbiol. 12, 235–246.

Buchanan, R.L., Phillips, J.G., 1990. Response surface model for predicting the effects of temperature, pH, sodium chloride content, sodium nitrite concentration and atmosphere on the growth of *Listeria monocytogenes*. J. Food Prot. 53, 370–376.

Fehlhaber, F., Kruger, G., 1998. The study of *Salmonella enteritidis* growth kinetics using Rapid Automated Bacterial Impedance Technique. J. Appl. Microbiol. 84, 945–949.

Hudson, J.A., 1993. Effect of pre-incubation temperature on the lag time of *Aeromonas hydrophila*. Lett. Appl. Microbiol. 16, 274–276.

Jansson, J.K., 2003. Marker and reporter genes: illuminating tools for environmental microbiologists. Curr. Opin. Microbiol. 6, 310–316.

Mackey, B.M., Kerridge, A.L., 1988. The effect of incubation temperature and inoculum size on growth of salmonellae in minced beef. Int. J. Food Microbiol. 6, 57–65.

McClure, P.J., Blackburn, C.W., Cole, M.B., Curtis, P.S., Jones, J.E., Legan, J.D., Ogden, I.D., Peck, M.W., Roberts, T.A., Sutherland, J.P., Walker, S.J., 1994. Modelling the growth, survival and death of microorganisms in foods: the UK Food Micromodel approach. Int. J. Food Microbiol. 23, 265–275.

Oblinger, J.L., Kraft, A.A., 1970. Inhibitory effects of *Pseudomonas* on selected *Salmonella* and bacteria isolated from poultry. J. Food Sci. 35, 30–32.

Oscar, T.P., 1998. Growth kinetics of *Salmonella* isolates in a laboratory medium as affected by isolate and holding temperature. J. Food Prot. 61, 964–968.

Oscar, T.P., 1999a. Response surface models for effects of temperature and previous growth sodium chloride on growth kinetics of *Salmonella typhimurium* on cooked chicken breast. J. Food Prot. 62, 1470–1474.

Oscar, T.P., 1999b. Response surface models for effects of temperature and previous temperature on lag time and specific growth rate of *Salmonella typhimurium* on cooked ground chicken breast. J. Food Prot. 62, 1111–1114.

Oscar, T.P., 1999c. Response surface models for effects of temperature, pH, and previous growth pH on growth kinetics of *Salmonella typhimurium* in brain heart infusion broth. J. Food Prot. 62, 106–111.

Oscar, T.P., 2000. Variation of lag time and specific growth rate among 11 strains of *Salmonella* inoculated onto sterile ground chicken breast burgers and incubated at 25 °C. J. Food Saf. 20, 225–236.

Oscar, T.P., 2003. Comparison of predictive models for growth of parent and green fluorescent protein-producing strains of *Salmonella*. J. Food Prot. 66, 200–207.

Oscar, T.P., 2005a. Development and validation of primary, secondary and tertiary models for predicting growth of *Salmonella typhimurium* on sterile chicken. J. Food Prot. 68, 2606–2613.

Oscar, T.P., 2005b. Validation of lag time and growth rate models for *Salmonella typhimurium*: acceptable prediction zone method. J. Food Sci. 70, M129–M137.

Oscar, T.P., Dulal, K., Boucaud, D., 2006. Transformation of *E. coli* K-12 with a high copy plasmid encoding the green fluorescent protein reduces growth: implications for predictive microbiology. J. Food Prot. 69, 276–281.

Rang, C., Galen, J.E., Kaper, J.B., Chao, L., 2003. Fitness cost of the green fluorescent protein in gastrointestinal bacteria. Can. J. Microbiol. 49, 531–537.

Rhodes, P., Quesnel, L.B., Collard, P., 1985. Growth kinetics of mixed culture in salmonella enrichment media. J. Appl. Bacteriol. 59, 231–237.

Ross, T., 1996. Indices for performance evaluation of predictive models in food microbiology. J. Appl. Bacteriol. 81, 501–508.

Ross, T., Dalgaard, P., Tienungoon, S., 2000. Predictive modeling of the growth and survival of *Listeria* in fishery products. Int. J. Food Microbiol. 62, 231–245.

Schmitt, R.E., Gallo, L., Schmidt-Lorenz, W., 1988. Microbial spoilage of refrigerated fresh broilers. IV. Effect of slaughtering procedures on the microbial association of poultry carcasses. Lebensm.-Wiss. u. -Technol. 21, 235–238.

Snary, E.L., Kelly, L.A., Davison, H.C., Teale, C.J., Wooldridge, M., 2004. Antimicrobial resistance: a microbial risk assessment perspective. J. Antimicrob. Chemother. 53, 906–917.

Stecchini, M.L., Ferraro, L., Caserio, G., 1988. Dynamics of *Salmonella* pre-enrichment in buffered peptone water. Microbiol. Aliments Nutr. 6, 367–371.

Thayer, D.W., Muller, W.S., Buchanan, R.L., Phillips, J.G., 1987. Effect of NaCl, pH, temperature, and atmosphere on growth of *Salmonella typhimurium* in glucose-mineral salts medium. Appl. Environ. Microbiol. 53, 1311–1315.

Thomas, H.A., 1942. Bacterial densities from fermentation tube tests. J. Am. Water Works Assoc. 34, 572–576.

Wijtzes, T., de Wit, J.C., Huis in't Veld, J.H.J., van't Riet, K., Zwietering, M.H., 1995. Modeling bacterial growth of *Lactobacillus curvatus* as a function of acidity and temperature. Appl. Environ. Microbiol. 57, 780–783.

Wright, J.G., Tengelsen, L.A., Smith, K.E., Bender, J.B., Frank, R.K., Grendon, J.H., Rice, D.H., Thiessen, A.B., Gilbertson, C.J., Sivapalasingam, S., Barrett, T.J., Besser, T.E., Hancock, D.D., Angulo, F.J., 2005. Multidrug-resistant *Salmonella typhimurium* in four animal facilities. Emerg. Infect. Dis. 11, 1235–1241.

Zwietering, M.H., Jongenburger, I., Rombouts, F.M., van 'T Riet, K., 1990. Modeling of the bacterial growth curve. Appl. Environ. Microbiol. 56, 1875–1881.