VARIATION OF LAG TIME AND SPECIFIC GROWTH RATE AMONG 11 STRAINS OF *SALMONELLA* INOCULATED ONTO STERILE GROUND CHICKEN BREAST BURGERS AND INCUBATED AT 25C[1]

THOMAS P. OSCAR[2]

United States Department of Agriculture
Agricultural Research Service
Microbial Food Safety Research Unit
1124 Trigg Hall
University of Maryland Eastern Shore
Princess Anne, MD 21853

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ABSTRACT

One strain of 11 serotypes or 11 strains of Salmonella, *which were isolated from the ceca of broilers, were surveyed for their growth kinetics on sterile ground chicken breast burgers incubated at 25C to determine the variation of lag time and specific growth rate. Growth curves, four per strain, were fit to a two-phase linear model to determine lag time (h) and specific growth rate (log_{10}/h). Repeatability of growth kinetics measurements for individual strains had a mean coefficient of variation of 11.7% for lag time (range: 5.8 to 17.3%) and a mean coefficient of variation of 6.7% for specific growth rate (range: 2.7 to 13.3%). Lag time among strains ranged from 2.2 to 3.1 h with a mean of 2.8 h for all strains, whereas specific growth rate among strains ranged from 0.3 to 0.38 log_{10} per h with a mean of 0.35 log_{10} per h for all strains. One-way analysis of variance indicated that lag time ($P = 0.029$) and specific growth rate ($P = 0.025$) differed slightly among strains.* S. *Haardt had a shorter ($P < 0.05$) lag time than* S. *Agona and* S. *Brandenburg, whereas the specific growth rate of* S. *Enteritidis was less than ($P < 0.05$) the specific growth rates of* S. *Typhimurium and* S. *Brandenburg. All other strains had similar lag times and specific growth rates. The coefficient of*

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[2]Author for correspondence: Tel: 410-651-6062; Fax: 410-651-6568; E-mail: toscar@mail.umes.edu.

variation among strains was 9.4% for lag time and 5.7% for specific growth rate. These results indicate that there were only minor differences in the lag times and specific growth rates among the strains of Salmonella *surveyed. Thus, the growth kinetic values obtained with one strain of* Salmonella *may be useful for predicting the growth of other strains of* Salmonella *for which data do not currently exist.*

INTRODUCTION

Although chicken may contain no or low numbers of *Salmonella* after processing (Waldroup *et al.* 1992), temperature abuse during distribution and meal preparation can result in rapid multiplication of *Salmonella* to infectious levels (Blaser and Newman 1982). Predicting the extent of *Salmonella* growth on chicken exposed to a particular time and temperature scenario requires knowledge of the strain(s) present and their growth potential. However, data regarding the variation of growth kinetics among strains of *Salmonella* found on/in chicken are limited. Thus, it is not clear how well data collected with one strain will predict the growth of other strains.

The variation of growth kinetics among strains of human bacterial pathogens is a consequence of interactions among the pathogen's environment and its genotype. In fact, recent surveys indicate that the variation of growth kinetics among strains of bacterial pathogens is influenced by the previous environment or source of isolation (Hudson 1992; Begot *et al.* 1997) and by the growth conditions used in the survey (Barbosa *et al.* 1994; Begot *et al.* 1997; Fehlhaber and Kruger 1998). In general, nonoptimal growth conditions are associated with greater variation of growth kinetics among strains than optimal growth conditions (Barbosa *et al.* 1994; Begot *et al.* 1997; Fehlhaber and Kruger 1998).

Previous surveys that assessed the variation of growth kinetics among strains of human bacterial pathogens were conducted in laboratory media (Barbosa *et al.* 1994; Begot *et al.* 1997; Fehlhaber and Kruger 1998). However, attachment of bacteria to solid surfaces, such as chicken, can alter gene expression and growth characteristics (Costerton *et al.* 1995). The effect of attachment on the variation of growth kinetics among strains of pathogens has not been quantified but may more closely reflect the situation on/in chicken and in other environments where the majority of bacteria are sessile rather than planktonic (Costerton *et al.* 1995). Consequently, the present study was undertaken to quantify the variation of growth kinetics among chicken strains of *Salmonella* in the sessile state by surveying their growth kinetics on sterile ground chicken breast burgers incubated at 25C.

APPROACH

Strains

The 11 strains and serotypes of *Salmonella* (Table 1) used in the current study were isolated from the ceca of broilers and were obtained from the microbiology laboratory of a local poultry company between March and August 1996 (Oscar 1998a).

Stock and Inoculum Cultures

Stock cultures of the 11 strains were maintained at -70C in brain heart infusion (BHI) broth (Difco Laboratories, Detroit, MI), pH 6.4 that contained 15% (volume/volume) glycerol. An inoculum culture for each growth curve in the survey was initiated by adding 5 µL of the appropriate strain's thawed stock culture to 5 mL of sterile BHI broth, pH 6.4, in a 25-mL Erlenmeyer flask sealed with a foam plug. After incubation for 23 h at 37C and 150 orbits per min, the inoculum culture (9.8 to 10.4 $\log_{10}$/mL) was serially diluted to 6.8 to 7.4 $\log_{10}$ per mL in sterile buffered peptone water and used to inoculate the surface of the sterile ground chicken breast burgers.

Preparation and Inoculation of the Sterile Ground Chicken Breast Burgers

Fresh chicken breast meat was purchased from a local supermarket on a weekly basis, cut into chunks, and ground twice through the 3/16 in. plate of a hand-powered meat grinder (Mode #32, The Sausage Maker, Inc., Buffalo, NY). Ten grams of the ground chicken breast meat were formed into a burger with a 1.2-cm^2-inoculation well. Burgers were sterilized by autoclaving at 121C for 18 min. The sterilized ground chicken breast burgers (6 g after autoclaving) were transferred under sterile conditions to sterile plastic petri dishes, placed in plastic ziploc bags, and stored at 4C for 24 to 72 h before use. Twelve burgers, representing 12 sampling times, were prepared for each growth curve.

Growth curves were initiated by inoculating the surface of the inoculation wells of a set of burgers, while cold, with 100 µL of the diluted starter culture using a sterile repeater pipette. After inoculation, the burgers were placed in ziploc bags, to prevent drying of the burgers during incubation, and placed in a 25C incubator. The initial number of *Salmonella* on the burgers was 4.8 to 5.4 $\log_{10}$ per burger. Warming of the burgers to 25C occurred during the course of the experiment to simulate how consumers may temperature abuse chicken (i.e., they would remove it from the refrigerator and place on the countertop at room temperature).

Survey Design

To assess the repeatability of the growth kinetic measurements for the 11 strains of *Salmonella*, four growth curves were conducted per strain for a total of 44 growth curves. The survey was conducted over an 11-week period at the rate of four growth curves per week and two growth curves per day. A new batch of chicken burgers consisting of 12 burgers per growth curve or 48 burgers was prepared each week. To avoid a systematic bias in the data due to unintentional and temporal changes in media, technique, meat and other factors, the four replicate growth curves for each strain were randomly assigned to the days of the survey.

Viable Cell Counts

At selected times after inoculation (Fig. 1), a chicken burger from the set of 12 for a given growth curve was homogenized (Model 400 Stomacher, Seward, London) for 2 min in 94 mL of sterile buffered peptone water. Undiluted and diluted samples of the homogenate were spiral plated (Whitley automatic spiral plater, Don Whitley Scientific Ltd., West Yorkshire, UK) onto BHI agar (1.5% agar; Difco Laboratories, Detroit, MI) and then incubated at 30C for 16 to 24 h before automated colony counting (Protos colony counter, Synoptics, Cambridge, UK). Viable cell count was expressed as $\log_{10}$ per mL of homogenate.

Curve-fitting

Viable cell counts were graphed as a function of sampling time. The resulting growth curves were iteratively fit (Prism® version 3.0, GraphPad Software, San Diego, Calif.) to a two-phase linear model (Buchanan *et al.* 1997):

$$Y = Y_o + \text{IF}\ [t \leq \text{LT}, 0, \text{SGR} * (t - \text{LT})]$$

where Y = the viable cell count ($\log_{10}$/mL of homogenate) at sampling time t (h), Y_o = the initial viable cell count ($\log_{10}$/mL of homogenate), LT = the lag time (h), and SGR = the specific growth rate ($\log_{10}$/h). The statement reads that IF $t \leq$ lag time then $Y = Y_o$ otherwise $Y = Y_o + [\text{SGR} * (t - \text{LT})]$.

Statistical Analysis

Data were analyzed by one-way analysis of variance to determine whether lag time and specific growth rate were affected ($P < 0.05$) by strain. When analysis of variance indicated a significant effect of strain means were compared using Tukey's multiple comparison test. Analysis of variance and means comparisons were performed using version 3.0 of Prism®. In addition, the coefficient of variation (CV) for lag time and specific growth rate within and among strains was calculated using the following formula:

$$CV = (SD/mean) * 100$$

where SD = standard deviation.

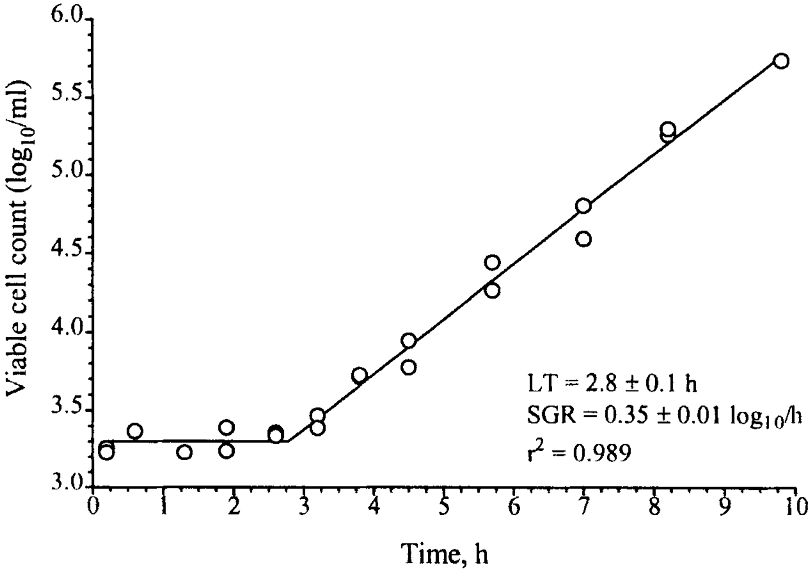

FIG. 1. RESULTS OF A TYPICAL GROWTH CURVE AND GROWTH-CURVE FIT FOR DETERMINING THE LAG TIME (LT) AND SPECIFIC GROWTH RATE (SGR) OF *SALMONELLA*
The results are for one replicate of *Salmonella* Mbanaka on sterile ground chicken breast burgers incubated at 25C. Growth data are graphed as $\log_{10}$ viable cell counts per mL of homogenate versus sampling time. Two dilutions of each homogenized sample were plated at each sampling time. In some cases only one of the plates had the appropriate number of colonies for automated counting. Thus, at some sampling times only one data point was available for curve fitting.

RESULTS

Figure 1 shows the results of a typical growth curve and growth curve-fit from the survey. The coefficient of determination (r^2), a measure of the goodness-of-fit of the data to the two-phase linear model, for the 44 growth curves was 0.971 ± 0.003 (mean ± SEM) with a range of 0.92 to 0.994.

TABLE 1.
VARIATION OF LAG TIME AND SPECIFIC GROWTH RATE VALUES AMONG 11 STRAINS OF *SALMONELLA* FROM THE CECA OF BROILERS WHEN THEY WERE INOCULATED ONTO STERILE GROUND CHICKEN BREAST BURGERS AND INCUBATED AT 25C

Strain	Isolate	*n*[1]	Lag Time Mean (h)	Lag Time CV[2] (%)	Specific Growth Rate Mean (log_{10}/h)	Specific Growth Rate CV[2] (%)
Salmonella Agona	s50	4	3.09	10.5	0.346	2.7
Salmonella Binza	s10	4	2.99	9.7	0.348	13.3
Salmonella Brandenburg	s20	4	3.07	11.9	0.376	4.3
Salmonella Enteritidis	s93	4	2.66	11.6	0.300	5.9
Salmonella Hadar	s91	4	2.73	5.8	0.356	9.0
Salmonella Harrdt	s116	4	2.20	12.2	0.332	8.3
Salmonella Indiana	s34	4	2.61	13.0	0.339	5.2
Salmonella Mbandaka	s7	4	2.75	9.4	0.358	8.3
Salmonella Senftenberg	s82	4	2.96	12.8	0.358	7.1
Salmonella Typhimurium	s83	4	2.79	14.3	0.365	5.2
Salmonella Worthington	s6	4	3.03	17.3	0.344	4.2
All strains		11	2.81	9.4	0.348	5.7

[1] *n* refers to the number of values that were used to calculate the summary statistics in the row. For individual strains, kinetic values from four replicate growth curves were used to calculate the mean kinetic value and its coefficient of variation. In contrast, for all strains, the mean kinetic values from the individual strains were used to calculate the mean kinetic values and their coefficients of variation

[2]CV refers to the coefficient of variation, which was calculated as follows: (standard deviation/mean)*100.

Repeatability of the lag time values among the four replicate growth curves conducted for each strain had a mean coefficient of variation of 11.7% for all strains and coefficients of variation that ranged from 5.8 to 17.3% for individual strains (Table 1). Repeatability of the specific growth rate values among the four replicate growth curves conducted for each strain had a mean coefficient of variation of 6.7% for all strains and coefficients of variation that ranged from 2.7 to 13.3% for individual strains (Table 1). Thus, specific growth rate values had slightly better repeatability than lag time values for individual strains.

Mean lag times for individual strains of *Salmonella* on sterile ground chicken breast burgers incubated at 25C ranged from 2.2 to 3.1 h with a mean lag time of 2.8 h for all strains (Table 1). One-way analysis of variance indicated that lag time was different ($P = 0.029$) among strains (Fig. 2). *S.* Harrdt had a shorter ($P < 0.05$) lag time than *S.* Agona and *S.* Brandenburg, whereas all other strains had similar lag times. The coefficient of variation for the mean lag times among the 11 strains of *Salmonella* was 9.4% (Table 1).

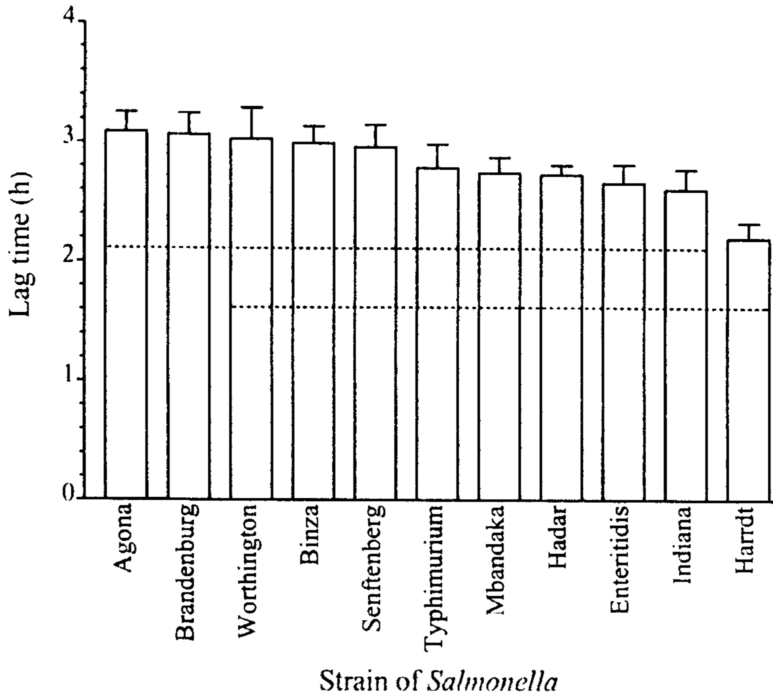

FIG. 2. VARIATION OF LAG TIME AMONG STRAINS OF *SALMONELLA* ON STERILE GROUND CHICKEN BREAST BURGERS INCUBATED AT 25C

Each bar is the mean ± SEM of four replicate lag time determinations. Bars (i.e., means) that are not connected by horizontal dotted lines are different at $P < 0.05$ as determined by one-way analysis of variance followed by Tukey's multiple comparison test.

Mean specific growth rates for individual strains of *Salmonella* on sterile ground chicken breast burgers incubated at 25C ranged from 0.3 to 0.38 $\log_{10}$ per h with a mean specific growth rate of 0.35 $\log_{10}$ per h for all strains (Table 1). One-way analysis of variance indicated that specific growth rate was different ($P < 0.025$) among strains (Fig. 3). *S.* Enteritidis had a lower specific growth rate than *S.* Typhimurium and *S.* Brandenburg, whereas all other strains had similar specific growth rates. The coefficient of variation for the mean specific growth rate among the 11 strains of *Salmonella* was 5.7% (Table 1). Thus, the variation of specific growth rate among the 11 strains of *Salmonella* surveyed was slightly less than the variation of their lag times.

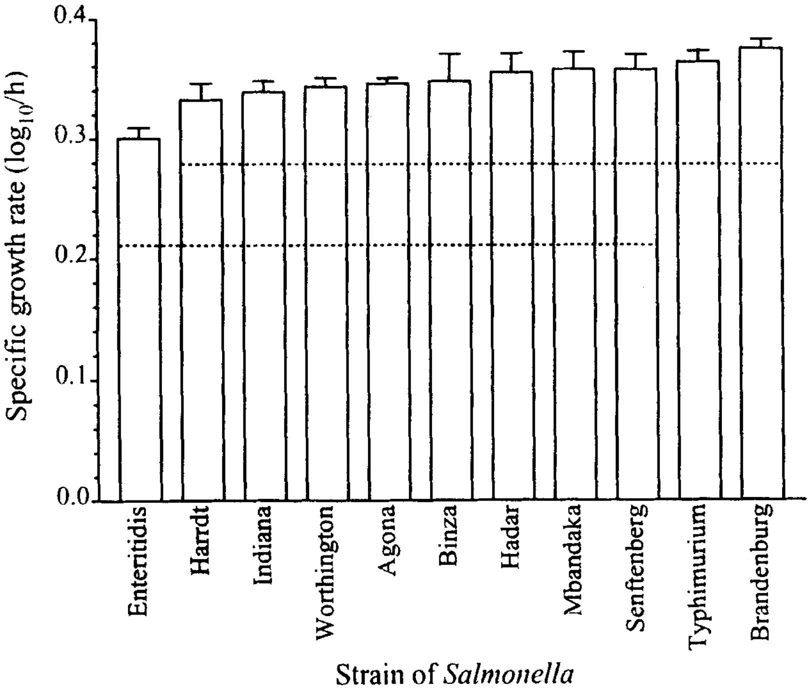

FIG. 3. VARIATION OF SPECIFIC GROWTH RATE AMONG STRAINS OF *SALMONELLA* ON STERILE GROUND CHICKEN BREAST BURGERS INCUBATED AT 25C

Each bar is the mean ± SEM of four replicate specific growth rate determinations. Bars (i.e., means) that are not connected by horizontal dotted lines are different at $P < 0.05$ as determined by one-way analysis of variance followed by Tukey's multiple comparison test.

DISCUSSION

Two statistical methods were used to evaluate the variation of growth kinetics among strains of *Salmonella* on sterile ground chicken breast burgers incubated at 25C. One-way analysis of variance was used to determine whether strain had an effect on lag time and specific growth rate and the coefficient of variation was used to quantify the variation of the growth kinetic values among the strains of *Salmonella* tested. Analysis of variance indicated that lag time and specific growth rate differed among strains. However, only three of the 11 strains surveyed had significantly different lag times and specific growth rates. Coupled with the coefficients of variation among strains of 9.4% for lag time and 5.7% for specific growth rate, these results indicate that the variation of growth kinetics among the strains of *Salmonella* surveyed was relatively small.

The results of the current survey agree with those of our previous survey (Oscar 1998b) and indicate that there are only minor differences in the growth kinetics among strains of *Salmonella* found on/in chicken. However, this conclusion must be tempered by the realization that greater variability of the growth kinetics among strains may have been observed had more strains been included in the current (11 strains) and previous (14 strains) surveys. The size of our surveys was constrained by the use of the labor-intensive viable cell counting method to enumerate *Salmonella* in the growth kinetic experiments. As an alternative, the size and scope of bacterial growth kinetic surveys can be increased by the use of automated spectrophotometric and impedance methods (Hudson 1992; Barbosa *et al.* 1994; Begot *et al.* 1997; Fehlhaber and Kruger 1998). However, these methods are limited to liquid samples and thus, they are not useful for assessing the variation of growth kinetics among strains growing in the sessile state on solid food, such as sterile ground chicken breast burgers.

The source of the isolate (Hudson 1992; Begot *et al.* 1997) influences the variation of growth kinetics among strains of bacterial pathogens. Using cluster analysis, Begot *et al.* (1997) showed that *Listeria monocytogenes* isolates from industrial sites were faster growing than isolates from meat products. Hudson (1992) found that *Aeromonas hydrophila* isolates from food were faster growing at low temperatures than isolates from clinical specimens and meat processing environments. In the current study, isolates from only one source, the ceca of broiler chickens, were used in the survey and this may have contributed to the low variation of growth kinetics among strains. However, in our previous survey (Oscar 1998b), we used isolates of *Salmonella* from a variety of environments in broiler operations (i.e., broiler ceca, broiler feed, broiler house litter swabs, broiler processing plant sludge, broiler carcass rinse, and broiler house beetles) and did not observe large variation of growth kinetics among strains.

The growth conditions used in a survey also influence the variation of growth kinetics among strains. In fact, Fehlhaber and Kruger (1998) reported that the coefficient of variation for generation time among 45 strains of *Salmonella* Enteritidis decreased from 22% to 4% as the temperature of incubation increased from the nonoptimal temperature of 9C to the optimal temperature of 37C. Begot *et al.* (1997) surveyed 66 strains of *Listeria monocytogenes* for their growth kinetics in laboratory medium under different combinations of temperature (10 or 37C), pH (5.6 or 7.0), and water activity (0.96 or 1.0) and found the greatest variation of lag time occurred at the nonoptimal growth condition of 10C, pH 7, and 0.96 water activity. In contrast, Barbosa *et al.* (1994) observed a similar variation of growth kinetics among 39 strains of *Listeria monocytogenes* in laboratory medium incubated at 4, 10, or 37C.

Although not reported in our published survey (Oscar 1998b), the coefficients of variation among the 14 strains of *Salmonella* grown in BHI broth (pH 6) incubated at the optimal temperature of 40C were 12.8% for lag time and 4.9% for specific growth rate. These coefficients of variation are similar to those reported in the current survey for the growth of *Salmonella* on sterile ground chicken breast burgers (pH 6) incubated at the nonoptimal temperature of 25C. Thus, our results agree with those of Barbosa *et al.* (1994) and suggest that growth conditions do not always alter the variation of growth kinetics among strains of bacterial pathogens.

An objective of assessing the variation of growth kinetics among strains of bacterial pathogens is to determine whether data collected with one strain are useful for predicting the growth of other strains found on/in that food. Great care should be taken in designing, conducting, and interpreting experiments that assess the variation of growth kinetics among strains. Improper experimental design and kinetic data with considerable variation due to large experimental error can obscure the attainment of the survey's objective. In the present survey, the coefficients of variation for growth kinetic values for individual strains, a measure of experimental error, were low because the experimental design was balanced and random (to avoid systematic bias in the data) and the quality of the kinetic data was high as indicated by the r^2 of the growth-curve fits. Thus, the current data provide an accurate assessment of the variation of growth kinetics among the strains of *Salmonella* tested and under the growth conditions used.

Although we are confident in the accuracy of the results of the current survey, there are two limitations of the current data that should be highlighted. First, the growth medium did not contain competing microorganisms and second, the sterile ground chicken breast burgers were incubated at only one temperature. Both of these limitations could affect the assessment of the variation of growth kinetics among strains. Our future approach to assessing the variation of growth kinetics among strains of bacterial pathogens will involve the use of nonsterile foods and marker (i.e., antibiotic resistant and fluorescent) pathogens to address the issue of competing microorganisms. In addition, a broad temperature range will be used to further address the issue of the effect of nonoptimal and optimal growth conditions on the variation of growth kinetics among stains of bacterial pathogens.

CONCLUSION

Only minor variation of lag times and specific growth rates were noted among 11 chicken strains of *Salmonella* growing on sterile ground chicken breast burgers incubated at 25C. The results suggest that growth kinetic values obtained with one strain of *Salmonella* may be useful for predicting the growth of other strains of *Salmonella* for which data do not currently exist.

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